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REVIEW ARTICLE

Fungal spores: A critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting

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Abstract

Fungal spores are ubiquitous in the environment. However, exposure levels in workplaces where mouldy materials are handled are much higher than in common indoor and outdoor environments. Spores of all tested species induced inflammation in experimental studies. The response to mycotoxin-producing and pathogenic species was much stronger. In animal studies, nonallergic responses dominated after a single dose. Allergic responses also occurred, especially to mycotoxin-producing and pathogenic species, and after repeated exposures. Inhalation of a single spore dose by subjects with sick building syndrome indicated no observed effect levels of 4×10^3 *Trichoderma harzianum* spores/m^3 and 8×10^3 *Penicillium chrysogenum* spores/m^3 for lung function, respiratory symptoms, and inflammatory cells in the blood. In asthmatic patients allergic to *Penicillium* sp. or *Alternaria alternata*, lowest observed effect levels (LOELs) for reduced airway conductance were 1×10^4 and 2×10^4 spores/m^3, respectively. In epidemiological studies of highly exposed working populations lung function decline, respiratory symptoms and airway inflammation began to appear at exposure levels of 10^5 spores/m^3. Thus, human challenge and epidemiological studies support fairly consistent LOELs of approximately 10^5 spores/m^3 for diverse fungal species in nonsensitised populations. Mycotoxin-producing and pathogenic species have to be detected specifically, however, because of their higher toxicity.

Keywords: *Hypersensitivity pneumonitis; irritation; occupational exposure limit; organic dust toxic syndrome; review; toxicity*

Contents

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Terms as used in this document

Term	Explanation
Actinomycetes	Bacteria that grow and replicate like filamentous fungi. Actinomycetes may produce large numbers of vegetative spores that are easily dispersed into the air.
Aerodynamic diameter	Diameter of a spherical particle with specific density equal to 1 g/cm^3 and the same sedimentation velocity as the actual particle.
Allergen	Antigen that can induce hypersensitivity reactions often mediated by inducing the production of IgE antibodies.
Allergic alveolitis	See Hypersensitivity pneumonitis.
Allergy	A hypersensitivity reaction initiated by immunological mechanisms.
Antigen	Molecule that may induce the production of immunoglobulin antibodies.

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Terms continued.

Term	Explanation
Asthma	Obstructive lung disease characterised by reversible attacks of airway obstruction following exposure to allergens and nonspecific irritants. In allergic asthma the response is mainly limited to specific allergens, and IgE antibodies to the allergen can be demonstrated. Non-allergic asthma does not seem to be IgE mediated.
Atopy	The ability of an individual to produce IgE antibodies after exposure to an allergen. Atopy can be tested by the presence of IgE antibodies to common allergens in serum or by skin prick tests with these allergens.
Colony-forming unit	A single microorganism or an aggregate of microorganisms that has grown into a countable colony by culture on a semisolid nutrient medium under controlled conditions.
Conidia	Asexual spores produced by fungi.
Culturable microorganisms	Microorganisms quantified by culture-based methods.
Eukaryotic organism	Organism with cell(s) that have well defined nuclei containing the genetic material.
Fluorochrome	Fluorescent dye used to visualise specific particles in the fluorescence microscope.
Fungi	Organisms belonging to the kingdom Mycota. Fungi can be divided into filamentous fungi that form hyphae and replicate by spores (moulds and mushrooms), and by budding of cells (yeast).
Genus	The next lowest taxonomic category; also called family.
Gram-positive	Bacteria are divided in two main groups based on a staining technique developed by Gram. The Gram-positive bacteria differ from Gram-negative bacteria by the structure and composition of the cell wall.
Heterophilic microorganisms	Microorganisms dependent on organic material from dead or living organisms for growth.
Hyphae	Branched multicellular filaments formed by filamentous fungi.
Hypersensitivity	Causes objectively reproducible symptoms, or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects.
Hypersensitivity pneumonitis	Alveolar and bronchiolar inflammation caused by inhalation of spores from fungi and actinomycetes, and other allergens. Acute attacks are similar to ODTS (see Organic dust toxic syndrome). Recurrent attacks may eventually progress into pulmonary fibrosis. IgG but not IgE can usually be detected. Also called allergic alveolitis.
Immunoglobulin E	Antibodies that are a part of the adaptive immune response. Specific and total immunoglobulin E can be detected in serum and play a role in allergic sensitisation and disease.
Immunoglobulin G	Antibodies that are a part of the adaptive immune response. Specific immunoglobulin G antibodies to fungal species in serum can be used as biomarkers of exposure but the precision is poor.
Impactor	Sampling device that collects particles according to inertial properties when the air stream is diverted after aspiration of the aerosol.
Impinger	Impactor that collects particles in a liquid.
Induced sputum	Sputum stimulated by inhalation of a saline aerosol.
Inhalation fever	See Organic dust toxic syndrome.
Job exposure matrix	A tool to estimate qualitative or quantitative exposure levels using job characteristics and exposure information.
Magic Lite test	A fluorometric enzyme immunoassay for measurement of IgE antibodies.
Mesophilic microorganisms	Microorganisms with optimal growth at temperatures between 15 and 30°C.
Metabolites	Intermediate compounds and products of metabolism.
Mycelium	All aggregated hyphae from a single organism.
Mycotoxins	Toxic compounds produced by a number of fungal species. Mycotoxins can be excreted in substrates and may also be found in spores from such species.
Organic dust toxic syndrome	Attacks of fever/chills with respiratory symptoms and malaise that develop 4–8 hours after massive exposure to some substances, e.g., metal fumes and organic particles. The symptoms resolve within a few days. IgG antibodies against fungi are often not detectable.

Terms continued on next page.

Terms continued.

Term	Explanation
Phadiatop	Combined detection of IgE against 10 common respiratory allergens by a radio-allergosorbent test. A positive test indicates the presence of atopy.
Precipitins	IgG antibodies detected in serum by the double-diffusion technique of Ouchterlony through formation of an antigen-antibody precipitate.
Primary metabolites	Metabolites essential for normal growth, development, and reproduction.
Proliferation	Rapid reproduction of microorganisms.
Psychrophilic microorganisms	Microorganisms that preferentially grow at temperatures below 15°C.
Secondary metabolites	Metabolites that are not essential for normal growth, development, and reproduction, but usually have important ecological functions. Examples are toxins, including antibiotics used in competition with other organisms, and pigments.
Sensitisation	Sensitisation is often understood as the presence of serum IgE antibodies to a specific allergen. However, hypersensitivity to allergen exposure in hypersensitivity pneumonitis patients is mediated by other mechanisms.
Spores	Sexual or asexual reproductive cells of fungi and actinomycetes (only asexual). Spores are metabolically inactive and tolerate environmental stress much better than vegetative cells. They are therefore important means for dispersion of organisms to other habitats.
Thermophilic microorganisms	Microorganisms that preferentially grow at temperatures above 30°C.
Toxic alveolitis	See Organic dust toxic syndrome.
Toxigenic microorganisms	Microorganisms that have the capability to produce toxins.
Viable organisms	Living organisms capable of germination, growing, and replication. Viable organisms include organisms that can grow on nutrient plates as well as living organisms not able to grow in culture, e.g., obligate parasitic organisms.

1. Introduction

Fungi are heterophilic organisms, i.e., dependent on dead or living organisms for their growth. They can quickly colonise all kinds of dead organic material and are together with bacteria the most important organisms that recycle organic material. Fungi are ubiquitous and have been estimated to comprise approximately 25% of the global biomass (Miller, 1992; Sorenson, 1999).

Fungi replicate by formation of sexual and asexual spores. Many species produce spores, which are easily dispersed into the air and can be transported over long distances, even across the globe; for an overview see Shinn et al. (2003). We therefore inhale substantial numbers of fungal spores from outdoor and indoor air. Exposure levels are highly variable because fungi can rapidly multiply when conditions for growth are favourable, and some species release their spores on specific times of the day. *Actinomycetes* are a group of bacteria that resemble the fungi in growth and replication, and may also be present in indoor and outdoor air.

Spores of fungi and actinomycetes have been recognised as occupational hazards; see reviews by Lacey and Crook (1988) and Malmberg (1991). In 1950–1980, several species were identified as causes of hypersensitivity pneumonitis (also called allergic alveolitis) in a number of occupations, including farmers, malt workers, and wood workers. Exposure levels in these occupations regularly exceed 10^6 spores/m^3 and can be as high as 10^{10} spores/m^3. These levels are much higher than levels usually found outdoors, which seldom exceed 10^4 spores/m^3. Fungal spore exposure has also been associated with indoor air problems, especially in damp buildings. Airborne concentrations in such environments may be higher than outdoors, but levels rarely exceed 10^4 colony-forming units/m^3 (cfu/m^3), which correspond to approximately 10^5 spores/m^3 (review by Levetin, 1995).

Fungal spores are complex agents that may contain multiple hazardous components. Health hazards may differ across species because fungi may produce different allergens and mycotoxins, and some species can infect humans.

The epidemiological and toxicological literature on health effects from occupational exposure to fungal spores has not been reviewed comprehensively before. The aim of this review is therefore to (1) critically evaluate the evidence of health effects associated with fungal spore exposure at the workplace; and (2) estimate no or lowest observed effect levels as a basis for health-based occupational exposure limits for fungal spores.

1.1. Delimitations

Delimitations are needed due to the complexity of the subject. Infectious diseases will not be addressed. Also, genetically altered fungi are omitted since limited information is available on occupational health risks. β(1→3)-Glucans, which have been studied as fungal agents (review by Douwes, 2005), are also excluded because they are not specific to fungi and can also be found in plants and bacteria. Mycotoxins

have been subject for review elsewhere, see IPCS/WHO (1979) and CAST (2003), since they are of great concern in the public health sector from the perspective of oral intake of contaminated food. However, mycotoxins present in spores will be considered as their presence may increase the toxicity of the spores. The actinomycetes are included because they have similar effects on human health as fungal spores and may also represent important health risks to working populations.

2. Species characterisation

The fungi comprise a large group of organisms including mushrooms, moulds, and yeasts. The fungi are placed in the kingdom Mycota, and most fungi relevant to human health belong to the division Amastigomycota. Fungi are traditionally identified by morphological characteristics of colonies and reproductive structures including spores, and conditions for growth in culture. These characteristics are compared to the characteristics of type species described in the literature and stored in type collections. The use of molecular biological methods is rapidly increasing, however. Important genera in occupational environments are shown in Table 1.

Many species have been described in the literature under different names, and have later been recognised as the same species. Table 2 aims at identifying species described in older publications cited in this review. The most recent names have been used in this document.

3. Biological and physical properties

General references to this topic are Gregory (1973), Al-Doory and Domson (1984), Burge (1989), and Green (2005).

3.1. Fungi

Fungi are eukaryotic organisms that lack chlorophyll and depend on other organisms for their supply of nutrients. Most fungi are saprophytic, i.e., live on dead organic material. Fungi play an important role in the ecosystem in the recycling of nutrients. However, as fungi can exploit all organic materials, they may also damage food, wood, and textiles as well as building materials in buildings with humidity problems. Fungi may even invade living organisms and infect plants, animals, and humans. Plant pathogenic fungi are of major concern for farmers as they cause significant damage to crops.

Filamentous fungi (moulds and mushrooms) grow as branched multicellular filamentous structures (hyphae) that collectively form the mycelium. Fungi need organic material, oxygen, and water for growth. As oxygen and organic material are readily available in most environments, access to water is usually the limiting factor. A water content above 12–15% in materials such as grain and wood is usually sufficient to sustain fungal growth, but some fungi can even grow in materials with lower water content if the relative humidity in the air is above 85%.

Temperature has a major influence on the growth of microorganisms. Most fungal and actinomycete species are mesophilic and show optimal growth at 15–30°C. Psychrophilic and psychrotolerant species grow at lower temperatures, e.g., *Cladosporium herbarum* can grow at temperatures down to –5°C. Thermophilic species have growth optima above 30°C. Fungi may also grow outside the optimal temperature range. However, exceedance of the higher temperature limit for growth may kill organisms, whereas temperatures below the lower growth limit are less lethal. *Aspergillus fumigatus* has a growth optimum close to the human body temperature and is the most important opportunistic infectious agent. pH conditions for growth are often broad, with optima around pH 6. However, few fungi grow below pH 3 or above pH 9.

3.2. The compost reaction

When organic material with a sufficient water content is stored at ambient temperature for prolonged time,

Table 1. Aerodynamic diameter (AED) of single and aggregated spores from fungi and actinomycetes released from culture plates.

Species	AED[a] (µm)	Reference
Fungi		
Aspergillus fumigatus	2.0-2.7	Pasanen *et al* (1991)
	1.9-2.2	Madelin and Johnson (1992)
	2.1-2.2	Reponen *et al* (1996)
Cladosporium cladosporoides	2.3-2.5	Madelin and Johnson (1992)
	1.7-1.9	Reponen *et al* (1996)
Penicillium brevicompactum	2.0-2.2	Reponen *et al* (1996)
	2.1-2.4	Reponen *et al* (1997)
Penicillium chrysogenum	2.6-3.0	Madelin and Johnson (1992)
Penicillium melinii	2.7-2.8	Reponen *et al* (1996)
Stachybotrys chartarum	4.5	Sorenson et al (1987)
Actinomycetes		
Micromonospora halophytica	1.3	Reponen *et al* (1998)
Streptomyces albus	0.9-1.9	Reponen *et al* (1997)
	0.85	Reponen *et al* (1998)
Thermoactinomyces vulgaris	0.57	Reponen *et al* (1998)

[a] Geometric mean.

Table 2. Working populations potentially exposed to fungal and actinomycete spores (Statistics Denmark 2006; Statistics Finland 2006; Statistics Iceland 2006; Statistics Norway 2006; Statistics Sweden 2006).

	Working population in thousands				
Occupation	Denmark 2005	Norway 2005	Sweden 2004	Finland 2005	Iceland 2005
Agriculture and forestry	65	59	36	116	7
Agriculture	61	55	23		
Forestry	4	4	13		
Food industry	72	44	63	nf	nf
Wood industry	14	9	36	nf	nf
Wood processing	7	4	40	nf	nf
Furniture	19	nf	44	nf	nf
Total working population	2 400	2 800	4 200	2 500	160

nf: information not found.

psychrophilic or mesophilic organisms may start to grow. The metabolic activity produces heat, which may raise the temperature in the material and modulate fungal growth depending on the growth optima of the microorganisms present. Proliferation of thermophilic species may raise the temperature even further. The maximum temperature that stored material may reach mainly depends on the original water content (Lacey & Crook, 1988). The material may eventually catch fire due to ignition of volatile organic compounds produced by the microorganisms (MVOCs). When nutrients are depleted, microbial growth slows down and temperature declines again. Water content, temperature, and storage time are therefore important determinants of the level and the complexity of the microbial contamination in organic materials.

3.3. Spores

Most fungi are adapted to aerial dispersion and replicate by nonmotile asexual and sexual spores. Fruiting bodies may grow from the mycelium of a single organism and produce asexual spores (Figure 1). Sexual reproduction involves fusion of the mycelia of two different mating strains and subsequent development of sexual spores. Yeasts grow as single cells and replicate by budding under wet conditions and do not become easily airborne. However, some yeast species have both cellular and mycelial growth and may produce airborne spores dependent on environmental conditions.

Both sexual and asexual spores are resting, metabolically inactive units surrounded by a thick wall that protects the organism from the environment. Spores can tolerate extreme physical conditions and may survive until conditions become favourable for growth. Many species liberate their spores into the air. As spores often have a small size they can stay airborne for long periods and be transported over large distances. Thus, spore formation is important for survival, and replication, as well as dispersion of the fungus.

Spore sizes range typically from 2 to 10 µm among species and vary even among spores from the same species. The physical diameter of spherical and smooth spores is probably similar to their aerodynamic diameter (AED), but spores with elongated shape and/or surface ornamentation have smaller AEDs than the diameter of a sphere with the same volume as the spore because of larger drag forces in air. It should be noted that airborne spores may have a smaller size than quoted in manuals, since these data usually refer to the size of hydrated spores observed with the light microscope, whereas spores rapidly desiccate when dispersed in air. The specific gravity may have some influence on the AED as well since the density of spores varies from 0.4 to 1.5 g/cm^3. Air humidity also has some effect on AED, as the AED increased by 11–27% at 100% relative humidity but no changes were observed at relative humidities ranging from 30% to 90% (Reponen et al., 1996). Thus inhaled spores may increase in size in the airways, which has some effect on the deposition of in the airways (Section 7.1), but it is not known how rapid fungal spores absorb water vapour as many spore types are hydrophobic.

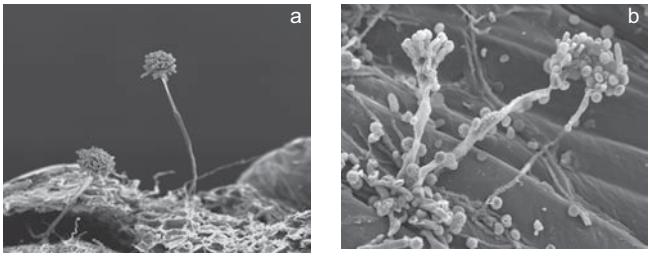

Figure 1. Fungal colonization of grass seeds. The micrographs show mycelium, fruiting bodies and spores from an *Aspergillus* (a) and a *Penicillium* species (b). Micrographs by Lene Madsø, National Institute of Occupational Health, Norway.

Spores may be released aggregated as chains or clumps that have larger AEDs than the single spores. The increase of the AED with aggregate size was relatively small, however (Reponen, 1995). Figure 2 shows some examples of spores and aggregates. AEDs of fungal spores generated from colonies and measured in the laboratory are shown in Table 3.

Spores from some genera such as *Fusarium* and *Stachybotrys* are produced in slimy aggregates, which are dispersed outdoors by raindrops (splash dispersal). The sticky spores may adhere and infect nearby plants or fall to the ground. Spores from these species can also be found airborne, however, and are probably released from plant material by mechanical friction during harvest and further handling of grain and straw.

Spores are often regarded as the most prevalent fungal particles that are airborne. However, recent experimental studies have demonstrated that spores as well as fragments that are smaller than spores can be released from fungal cultures by an air stream. This has been shown for several species, e.g., A*spergillus versicolor, Cladosporium cladosporoides, Penicillium melinii, Streptomyces albus, Trichoderma harzianum*, and *Ulocladium* sp., but not *Penicillium chrysogenum* (Kildesø et al., 2000, 2003; Górny et al., 2002, 2003; Madsen et al., 2005). Previously Sorenson et al. (1987) reported the liberation of hyphal fragments from cultures of *Stachybotrys chartarum*. The fungal origin of these fragments is supported by the detection of fungal antigens in the particle size fraction that only contained fragments (Górny et al., 2002), and by the staining of fragments with a DNA/RNA fluorochrome (Madsen et al., 2005). Recent environmental and occupational studies indicate that even larger hyphal fragments may have been overlooked (Green et al., 2005; Halstensen et al. 2007).

3.4. Composition of the fungal cell wall

Mycelial fungal and yeast cell walls are relatively thin, typically 0.2 µm. The main constituents vary in composition between fungi of different taxa. The cell wall is made up of a matrix containing β(1→3)-, α(1→3)-, and β(1→6)-glucans, glycoproteins, and lipids, reinforced by chitin (polyacetyl glucoseamine) or cellulose fibres. Pigments such as melanin may also be present. The lipid constituent ergosterol is commonly present and is used as a chemical marker for fungal mass (Section 5.1.4). The fungal cell wall has an outer

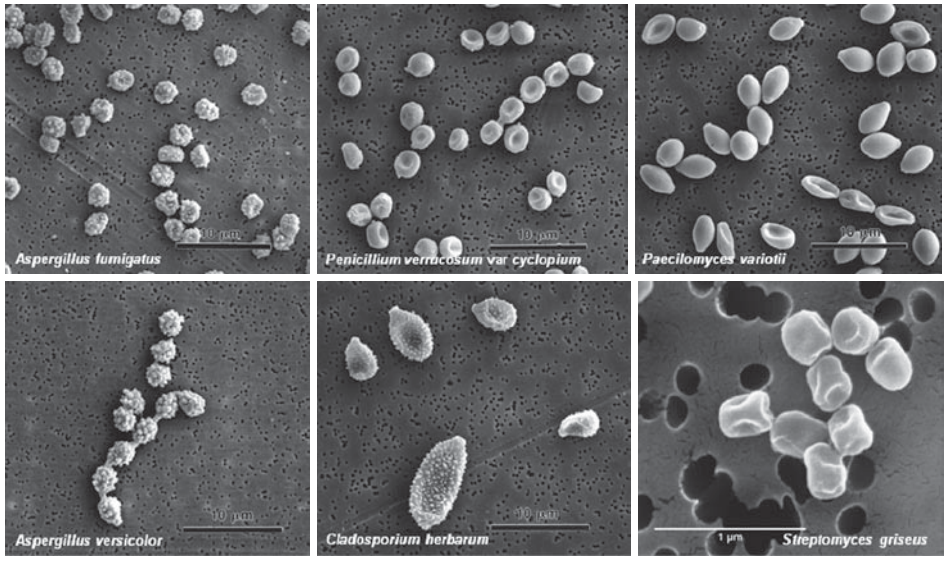

Figure 2. Single spores and spore aggregates with different morphology and size from fungal species commonly occurring in high exposure and common indoor environments. Micrographs of fungal spores have the same magnification while the magnification of the actinomycete spores (*S. griseus*) is approximately 15× higher. Micrographs by Katrin Karlsson, National Institute of Working Life, Sweden.

Table 3. Aerodynamic diameter (AED) of single and aggregated spores from fungi and actinomycetes released from culture plates.

Species	AED[a] (µm)	Reference
Fungi		
Aspergillus fumigatus	2.0–2.7	Pasanen et al. (1991)
	1.9–2.2	Madelin & Johnson (1992)
	2.1–2.2	Reponen et al. (1996)
Cladosporium cladosporoides	2.3–2.5	Madelin & Johnson (1992)
	1.7–1.9	Reponen et al. (1996)
Penicillium brevicompactum	2.0–2.2	Reponen et al. (1996)
	2.1–2.4	Reponen et al. (1997)
Penicillium chrysogenum	2.6–3.0	Madelin & Johnson (1992)
Penicillium melinii	2.7–2.8	Reponen et al. (1996)
Stachybotrys chartarum	4.5	Sorenson et al. (1987)
Actinomycetes		
Micromonospora halophytica	1.3	Reponen et al. (1998)
Streptomyces albus	0.9–1.9	Reponen et al. (1997)
	0.85	Reponen et al. (1998)
Thermoactinomyces vulgaris	0.57	Reponen et al. (1998)

[a]Geometric mean.

layer mainly consisting of glycoproteins that determine the antigenic properties of the cell. The cell wall has also been shown to contain high levels of fungal allergens (Bouziane et al., 1989). The secretion of hydrophobins (small, hydrophobic proteins) from growing hyphae has more recently been described. The hydrophobins coat the outer surface of aerial hyphae and spores. The hydrophobin coating allows hyphae to attach to hydrophobic surfaces and hosts, and plays an important role in fungal infections; review by Wessels (1996). The hydrophobins have been shown to be allergenic (Weichel et al., 2003). Pathogenic species may have an extracellular mucous layer on the cell surface that contains adhesins, molecules that allows the fungus to adhere to the host/substrate, and enzymes that may help to invade the host or liberate nutrients. Spores have much thicker cell walls than mycelia. The outer layer is hydrophobic and contains lipids and proteins. The inner cell wall is similar to the mycelial wall and consists of a β(1→3)-glucan matrix reinforced with chitin fibrils. The spore wall may liberate antigens that may induce allergic responses after deposition in the respiratory system (Cole & Samson, 1984; Moore-Landecker, 1996; de Nobel et al., 2001). All these components are primary metabolites as they are essential for the survival of the microorganisms.

3.5. Actinomycetes

The actinomycetes are Gram-positive bacteria that mimic fungi as they grow as branched cell chains and reproduce by production of asexual spores. Spores from actinomycetes are smaller than fungal spores, typically 0.5–1.5 µm, but otherwise have similar shape and surface characteristics. Most species are mesophilic and are abundant in soil. They usually require a higher water content for growth than fungi. Thermophilic species such as *Thermoactinomyces* sp. and *Saccharopolyspora rectivirgula* grow at higher temperatures than the fungi, up to 60-65°C, and may dominate the microbial biota in composting material at temperatures exceeding 50°C.

3.6. Metabolites

Primary metabolites are constituents of microorganisms that are essential for survival of the organism, such as the cell wall components (described in Section 3.4). Microorganisms may also produce secondary metabolites, which are compounds that are not vital but may be beneficial to the organism, e.g., mycotoxins, which are probably used in the competition with other organisms, and enzymes. MVOCs are formed during both the primary and the secondary metabolism. These metabolites are briefly described below.

Mycotoxins are potent toxins that a number of toxigenic fungi can excrete in substrates they colonise, and release in spores (Sorenson, 1987). Mycotoxins have mainly been studied as contaminants of food and feed; review by CAST (2003). Especially aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* have obtained much attention because of their strong carcinogenic and toxic properties. Other important mycotoxins are the trichothecenes produced by *Fusarium* and *Stachybotrys* species, fumonisins and zearalenone produced by *Fusarium* species, and ochratoxin A produced by *Aspergillus ochraceus* and *Penicillium verrucosum*. Many mycotoxins are immunotoxic. However, the trichothecene mycotoxins are immunostimulating at lower doses (review by Bondy & Pestka, S2000).

The production of mycotoxins strongly depends on growth conditions. For example, a toxic strain of *Stachybotrys chartarum* did not produce satratoxins when grown on pine or on other materials at a relative humidity below 84% (Nikulin et al., 1994).

Some mycotoxins are antibiotics, i.e., they are highly toxic to bacteria, but have low toxicity in humans. The antibiotics include penicillins produced by *Penicillium chrysogenum* and cephalosporins produced by *Cephalosporium* species. A review of occupational health risks from penicillins was recently published by the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) (Moore & Nygren, 2004).

Many fungal enzymes are glycopeptides with allergenic properties. Fungi may therefore represent a risk of allergic disease (Kurup et al., 2000). Enzymes are present in spores, and are released in greater amounts during germination of spores (Mitakakis et al., 2001; Green et al., 2003) and mycelial growth (Ibarrola et al., 2004). In the food industry, purified fungal enzymes such as α-amylase from *Aspergillus oryzae* (Houba et al., 1996), cellulase, xylanase, phytase, and α-amylase from *Trichoderma reesei* (Vanhanen et al., 1997), and phytase from *Aspergillus niger* (Doekes et al., 1999) have been shown to be potent allergens.

Fungi may produce a variety of allergens. For example, an international allergen nomenclatural committee has approved 17 different allergens from *Aspergillus fumigatus*, 6 allergens from *Alternaria alternata,* and 10 allergens from *Cladosporium herbarum*. A smaller number of allergens (1–3) have been recognised in three *Aspergillus* and four *Penicillium* species (Kurup *et al.*, 2000).

A few fungal species are commonly recognised as aeroallergens. Species from the genera *Cladosporium*, *Alternaria,* and *Aspergillus* are most often involved in fungal allergy and have been studied in detail (reviews by Hoffman 1984; Kurup et al., 2000) and these species are also most prevalent in outdoor air (Lacey, 1981). However, several other species and genera have been related to asthma in asthmatic patients (Kurup et al., 2000) and in working populations (Lacey & Crook, 1988), but they are not commonly included in allergy tests, probably because their occurrence in outdoor air is low (Lacey, 1981). It is therefore possible that other species than *Cladosporium*, *Alternaria,* and *Aspergillus* spp. may cause fungal allergy in working populations because they are often exposed to a fungal biota that is different from outdoor air.

Mainly spores have been studied as respiratory allergens as they have been regarded as the most prevalent airborne fungal particles. This may be a simplification, as hyphae (Section 3.1) have been given little attention. Furthermore, fungi may excrete allergens that can be present in other particles as well (Reed, 1985). The increased production of allergens in the germination phase suggests that viable spores may be more allergenic than dead spores if germination occurs in the respiratory tract (Mitakakis et al., 2001; Green et al., 2003), and Sercombe et al. (2006) recently demonstrated that germinating spores in the nasal cavity of healthy subjects were common. Górny et al. (2002) showed in an experimental study that fungal fragments smaller than spores were liberated from sporulating cultures, and that these fragments had high antigenic activity.

Microorganisms produce a large number of MVOCs. These compounds include alcohols, aldehydes, ketones, esters, terpenes, and organic sulphur and nitrogen compounds. MVOCs are mainly regarded to be side-products of the primary metabolism during synthesis of DNA and amino and fatty acids. However, the division between primary and secondary metabolism is not absolute and it is likely that MVOCs are formed during both (Korpi et al., 2006). The production of MVOCs strongly depends on the substrate and environmental conditions and many compounds denoted MVOCs may also originate from nonmicrobial sources. A criteria document on MVOCs has recently been published by NEG (Korpi et al., 2006).

3.7. Summary

Fungal spores are very different from chemical agents as spores may contain multiple components such as allergens, antigens, polysaccharides such as the β(1→3)-glucans, and mycotoxins. Many species produce spores that differ not only with respect to composition but also morphology (size, shape, and aggregation), which may influence the deposition in the respiratory system. Fungi are living organisms that multiply rapidly under favourable conditions and may colonise organic material when water is available in sufficient quantity. Many fungi produce large numbers of spores that are adapted to aerial dispersion. High-exposure situations are therefore often related to handling of mouldy material. Fungi may produce mycotoxins, MVOC, and enzymes, which often are allergenic. Actinomycetes are Gram-positive bacteria for which the growth and sporulation resemble that of filamentous fungi, but they have smaller spores. Recently described phenomena are the liberation of small fragments from fungal colonies and the presence of airborne hyphal fragments.

4. Occurrence, production, and use

Fungi are found in practically all habitats. Many species liberate their spores into the air, and these can be transported over long distances by air currents because of their small

size. Fungal spores are therefore ubiquitous in outdoor air, and can also be found indoors as they enter the indoor environment with outdoor air. Indoor spore levels may increase by unintended fungal growth or by handling of mouldy materials.

Outdoor levels vary between regions and seasons over a range of <20 to >10^5 cfu/m^3; see review by Gots et al. (2003). Outdoor levels are highest in warm regions and in the summer and autumn in temperate regions, and lowest in the winter in subarctic regions, e.g., <10^2 cfu/m^3 or <10^3 spores/m^3 in Finland (review by Pasanen, 1992).

Fungal spores in outdoor air enter the indoor environment by natural and mechanical ventilation but spore levels in common indoor environments are usually lower since spores are removed by filtration and/or they settle due to lower air velocities indoors. In spite of this, fungi in office buildings and houses have frequently been studied as a health risk. Fungi may grow on many types of building materials if the humidity of the air and/or in the materials is sufficient.

A typical cause of fungal growth is unattended water leakage. After the energy crisis of the 1970s, buildings in temperate regions were built more tightly and with higher thermal isolation to reduce energy costs. Lower air exchange rates and inadequate construction methods repeatedly resulted in condensation of water and microbial growth in the walls, floors, and roofs. Although the presence of fungi can often be demonstrated in damp buildings, the levels of airborne fungi indoors are mostly similar or only moderately elevated compared to outdoor levels, and rarely exceed 10^4 cfu/m^3. Measurements of airborne fungi may therefore fail to detect indoor fungal growth. Specific quantitation of *Penicillium, Aspergillus, Stachybotrys chartarum,* and yeasts may improve such assessments as these fungi are indicative of humidity problems while outdoor levels are often low; see review by Levetin (1995). In subarctic climates, assessments of fungal growth in buildings are preferably performed in the winter due to low outdoor levels (Section 5.2).

Although the assessment of mould problems by measurements of airborne fungi is problematic, it is interesting that remediation of humidity problems in buildings seems to reduce building-related health problems.

Fungal spore levels can be much higher at workplaces where the presence of fungi is related to production, and the contribution from outdoor levels usually is negligible. Fungi are used in the food industry, e.g., for production of dairy products, alcoholic beverages, bread, and soy sauce; in the biotechnological industry for production of citric acid, antibiotics, and enzymes; and for composting of plant debris and the organic fraction of domestic waste. Fungi can also be produced as an end-product, e.g., baker's yeast and mushrooms. Exposure levels often exceed 10^6 spores/m^3 and can be as high as 10^{10} spores/m^3 (reviews by Lacey & Crook, 1988; Malmberg, 1991). High levels may occur when fungi colonise organic materials unintentionally. These conditions are normally avoided because fungal contamination may lead to economical losses, and exposure levels are generally much lower. Important fungal and actinomycete genera in such work environments are shown in Table 1 (from review by Lacey & Crook, 1988).

5. Measurement methods and strategies for assessment of workplace exposure

5.1. Measurement methods for airborne fungal and actinomycete spores

Measurement methods for bioaerosols have been reviewed by several authors, see for example Eduard and Heederik (1998) and Douwes et al. (2003). These methods are therefore only shortly discussed below.

Most measurement methods for microorganisms estimate different entities. At present there is no general agreement on how fungal spore exposure should be assessed. The demonstration of fungal particles other than spores such as hyphae and smaller fungal fragments further complicates the assessment of fungal exposure, and their importance is not yet clear.

Spores can be collected by impaction on semisolid nutrient plates or glass slides, by impaction in liquid (impingers) and on filters. Most sampling instruments are stationary except filters, which are well adapted for personal sampling. Filter sampling is generally preferred since personal sampling is straightforward and can be carried out according to criteria for health-related size fractions (CEN, 1993). Filter samples can be analysed by culture-based and a variety of nonculture methods.

In epidemiological studies, mainly culture-based and microscopic methods have been used and both methods have shown exposure-response relationships in the majority of the studies (Eduard, 2003). However, culture-based methods suffer from several well-documented errors, and the cfu is at best a semiquantitative measure. The main advantages of culture-based methods are that microorganisms can be identified, and that the detection limit is lower than with microscopic methods. This is particularly important in common indoor environments. The best measurement strategy at present seems to be to combine culture-based methods for identification of species with nonculture methods for quantification of spore exposure. Molecular biological methods probably have the greatest potential for quantification of microbial exposure. However, these methods are at present mostly developed for qualitative assessment of fungi (Williams et al., 2001; Wu et al., 2002; Zeng et al., 2004).

5.2. Measurement strategies

Exposure assessment of fungal spores in highly contaminated work environments is similar to that of chemical agents. Personal sampling is preferred. This is straightforward for nonviable methods and even possible for some culture-based methods. There is no general understanding of the relevant sampling time, however. In environments where the exposure arises from contamination of handled materials, exposure levels may show high variability even

within days (Levy et al., 1999). Collection of samples over extended periods will reduce this variability and reduce the number of samples that have to be analysed. At present the commonly used 8-hour sampling time seems appropriate in the absence of better information on health relevant sampling duration.

Exposure levels in common indoor environments without apparent sources of fungal exposure, e.g., in offices, are much lower than in environments where mouldy material is handled. Fungal spore levels in common indoor air are expected to be similar or lower than outdoors because fresh air is often filtered before entering the ventilation system. Even in damp buildings, spore levels are often lower than outdoors. Nevertheless, indoor air problems seem to be related to moisture damage and fungal growth in buildings (Bornehag et al., 2001) and recognition of such damage is important for remediation of the problem. Inspections of buildings for fungal contamination and/or humidity problems by competent investigators and analysis of settled dust, floor dust, and building material samples for the presence of fungi have therefore been recommended (AIHA, 1993; ACGIH, 1999; Health Canada, 2004; Institute of Medicine, 2004). If such inspections are negative, airborne fungi can be measured to detect fungal growth in the building that is hidden from observation, e.g., within walls or ventilation systems. Indoor levels must be compared to outdoor levels to evaluate if concentrations of fungal species that adapted to proliferation indoors are elevated. As fungal levels in outdoor air can be substantial, especially in the summer and autumn, comparisons are based on species that are rare in outdoor air. It is important that outdoor fungal levels are measured several times a day because of their high within-day variability (Spicer & Gangloff, 2005). It is an advantage in subarctic regions to evaluate fungi in common indoor environments in the winter when outdoor fungal levels are low. This strategy is adopted in Finland (Reponen et al., 1992).

5.3. Conclusions

At present fungal spores can only be reliably quantified by microscopic counting. Viable as well as nonviable spores are counted but cannot be discriminated. Culturable fungi can be quantified by culture-based methods but only semiquantitatively as spore aggregates are counted as single colonies and also mycelium is detected. However, many factors influence the results, and results obtained by different methods or even by the same method in different environments are difficult to compare. The main advantages of culture-based methods are the possibility to identify fungal species, and higher sensitivity compared to microscopic counting.

Other methods may also be used to detect fungal agents, including ergosterol, glucans, antigens, and allergens, which may indicate the presence of fungal particles. None of these methods discriminate between spores and mycelium. Results therefore cannot be interpreted directly as spore levels. The detection of immunoglobulin G (IgG) antibodies can be useful as a biomarker of exposure in case studies of hypersensitivity pneumonitis where exposure data are not available.

The assessment of fungi in common indoor environments often has a different focus than in highly contaminated work environments where fungal exposure represents a risk for respiratory disease. The detection of water-damage and fungal growth is the main goal of many investigations in buildings, and measurement of airborne fungi is only one of several methods that can be applied for this purpose. In such studies, culture-based methods are commonly used for detection of airborne fungi because marker organisms can be identified at low levels. However, in epidemiological studies of indoor populations, culture-based methods have serious weaknesses.

6. Occupational exposure data

6.1. Highly contaminated environments

Occupational environments with high levels of exposure to fungi are found in agriculture, wood and food industry, and waste handling. Bioaerosol exposure at such workplaces is usually complex and fungi and actinomycetes comprise only some of the agents present. However, in some environments fungal spores are the main component of the bioaerosol and the exposure can even be limited to one or a few species. In such environments, specific organisms have been associated with hypersensitivity pneumonitis and asthma (Table 4).

Table 4. Workplaces in which fungi and actinomycetes have been associated with hypersensitivity pneumonitis and asthma; summarised from Lacey and Crook (1988).

Workplace	Source	Species
Agriculture	Mouldy hay	*Saccharomonospora viridis* *Saccharopolyspora rectivirgula* *Thermoactinomyces vulgaris* *Aspergillus umbrosus*
	Grain	*Aspergillus fumigatus*
	Grain (maize)	*Aspergillus flavus*
	Straw	*Aspergillus versicolor*
	Mushroom compost	*Saccharomonospora viridis* *Saccharopolyspora rectivirgula* *Thermoactinomyces vulgaris* *Aspergillus fumigatus*
	Mushrooms	*Lentinus edodus* *Pleurotus ostreatus*
Dairy	Cheese	*Penicillium camembertii* *Penicillium rockeforti* *Penicillium casei*
Brewery	Malted barley	*Aspergillus fumigatus* *Aspergillus clavatus*
Citric acid fermentation	Culture fluid	*Aspergillus niger*
Compost	Compost	*Aspergillus fumigatus*
Cork industry	Cork	*Penicillium glabrum*
Sugar mill	Bagasse	*Thermoactinomyces vulgaris* *Thermoactinomyces sacchari*
Tobacco factory	Tobacco	*Aspergillus fumigatus*
Wood industry	Maple bark	*Cryptostroma corticale*
	Coniferous wood	*Rhizopus microsporus* *Penicillium* spp.

When fungal colonisation occurs unintentionally, high spore concentrations can be generated when mouldy materials are handled. Work processes, preventive measures, and task performance further influence exposure levels. Exposure levels may therefore show large variability. This is clearly seen in farm work, where exposures during some tasks may differ by more than 4 orders of magnitude (Table 5).

Many exposure studies of nonagricultural occupations have been carried out. The number of epidemiological studies including quantitative exposure assessments is much smaller, however. The exposure data from these epidemiological studies are summarised in Table 6 as they are of greater importance for the present review. Exposure levels often exceed 10^4 cfu/m^3 or 10^5 spores/m^3.

Table 5. Exposure levels of culturable and countable fungi and actinomycetes during farm work (Eduard, 1997).

		Exposure range[a,b]							
Task	Agent	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Handling of									
grain	Fungi			<----------	----------	----------	----------	---------->	
hay	Fungi			<----------	----------	---------->			
	Fungi						<...>		
	Actinomycetes							<...>	
straw	Fungi			<----------	----------	----------	---------->		
compost	Actinomycetes						<.....	.....>	
Animal tending									
dairy/cattle	Fungi			<----------	----------	----------	---------->		
	Fungi					<.....	.....>		
	Actinomycetes					<.....	.....>		
swine	Fungi		<----------	----------	---------->				
poultry	Fungi		<---	--->					

[a]Dashed lines show colony-forming units/m^3 (cfu/m^3) and dotted lines spores/m^3.
[b]One cfu/m^3 corresponds approximately to 10 spores/m^3.

6.2. Common indoor environments

Fungal levels in common indoor environments without fungal problems are much lower than in the highly contaminated environments described above. A review of 10 studies including noncomplaint commercial buildings showed average levels of 17–1200 cfu/m^3 and 610–1000 spores/m^3, which amounted to 6–120% of outdoor levels (Gots et al., 2003). Additional studies of office buildings typically show fungal levels below 10^3 cfu/m^3, rarely exceeding 10^4 cfu/m^3 (Table 7). These data are difficult to interpret as exposure data because measurements in indoor environments are mainly performed using stationary sampling and culture-based analysis. Their accuracy is also poor because of poor analytical precision, short sampling duration, and variable culturability of the collected fungi, (Eduard & Heederik, 1998).

The main source of fungi in office environments is outdoor air. As outdoor air often is filtered before it enters the ventilation system and fungi settle due to lower air velocities in buildings than outdoors, common indoor fungal levels are expected to be lower than levels in outdoor air. This is also observed in the studies summarised in Table 7. In buildings with fungal growth levels indoors may be somewhat higher than outdoors but differences are usually small. Spores are easily dispersed by heating, ventilation, and air-conditioning systems, and contamination of such systems is an important cause of increased indoor fungal levels (Morey et al., 1984). An important activity that may contaminate indoor environments is the remediation of water-damaged building materials. Fungal levels may then exceed 10^5 cfu/m^3 (Rautiala et al., 1996).

In nonproblem indoor environments, outdoor fungi dominate, with *Cladosporium* and to some extent *Alternaria* and *Penicillium* as the dominating species. In buildings with fungal problems, levels of *Penicillium* and to some extent *Aspergillus* and yeasts are more often elevated compared

Table 6. Exposure to fungi and actinomycetes in epidemiological studies of highly exposed working populations.

			Exposure		
Population	Method	Agent	Culturable organisms cfu/m^3, range	Countable spores spores/m^3, range	Reference
Farmers	Personal sampling	Fungi		0–2×10^7	Eduard et al. (2001)
Farmers	Personal sampling, job exposure matrix[a]	Fungi		0–4×10^7	Eduard et al. (2004)
Sawmill workers (wood trimmers)	Personal sampling	Fungi	1×10^2–4×10^6		Hedenstierna et al. (1986)
Sawmill workers (wood trimmers)	Personal sampling	Fungi	3×10^{3b}	1×10^{5b}	Dahlquist et al. (1992)
Sawmill workers (wood trimmers)	Personal sampling	Fungi		4×10^5–2×10^7	Eduard et al. (1994)
Saw-/chip mill workers	Personal sampling	Fungi	3×10^3–7×10^{4c}		Alwis et al. (1999)
Joinery workers	Personal sampling	Fungi	4×10^3–2×10^{4c}		Alwis et al. (1999)
Waste collectors	Personal sampling	Fungi Actinomycetes		0–2×10^6 0–1×10^6	Heldal et al. (2003a, 2003b)

cfu: colony-forming units.
[a]Job exposure matrix: exposure estimated from information on performed tasks and other determinants, which had been validated by personal exposure measurement in a subset of the workers.
[b]Median.
[c]Range of arithmetic means.

Table 7. Culturable airborne fungi in office buildings.

Environment	Fungal species (in ascending prevalence order)	Total fungal levels, cfu/m^3	Reference
4 buildings *California, USA*	*Cladosporium*; nonsporulating fungi, *Aspergillus/Penicillium*	5–420 (15–35% of outdoor levels)	Schillinger et al. (1999)
84 randomly selected buildings *throughout USA*	Nonsporulating fungi, *Cladosporium*; *Penicillium*; yeasts; *Aspergillus*	0–4 000 (in 5% of the buildings were indoor levels > outdoor levels)	Womble et al. (1999)
4 buildings, 21 offices, sampled 1 year *Boston, Massachusetts, USA*	Nonsporulating fungi, *Penicillium*; *Cladosporium*; yeasts; *Aspergillus*	1–620	Chao et al. (2002)
1700 buildings (46% office), inspected because of complaints, water damage or fungal growth *throughout USA*	*Cladosporium*; *Penicillium*; non-sporulating fungi, *Aspergillus*	1–>10 000 82 (median indoors) 540 (median outdoors)	Shelton et al. (2002)
2 buildings without fungal problems, 6 buildings with fungal problems *continental, USA*	*Penicillium*	16–280 (11–86% of outdoor levels) 150–>3000 (<18–170% of outdoor levels)	Morey (1999)
15 buildings without preceding selection *Silesia, Poland*	*Penicillium*	50–1700 (summer) 18–110 (winter)	Pastuszka et al. (2000)
3 nonproblem offices, 2 offices with health complaints, 5 offices with fungal but no health problems *Prague, Czech Republic*	Not specified	nd–20 nd–70 190–330	Klanova and Drahonovska (1999)
1 air-conditioned building, 1 naturally ventilated building *Paris, France*	*Penicillium*; *Cladosporium*	17 (arithmetic mean, 4% of outdoor levels) 210 (arithmetic mean, 44% of outdoor levels) range 32–1 100	Parat et al. (1997)
28 randomly selected day-care centres *Taipei, Taiwan*	*Penicillium*; *Cladosporium*; yeasts; *Aspergillus*	1 200 ± 3.0 (geometric mean ± geometric standard deviation) (120% of outdoor levels)	Li et al. (1997)

cfu: colony-forming units; nd: not detected.

to outdoor air. Very humid conditions favour growth of *Stachybotrys chartarum.*

Levels of culturable fungi are much lower than fungi counted by microscopical techniques. Russell et al. (1999) found that culturable fungi amounted to 9% of counted spores in dwellings. This is also found in comparative studies in highly contaminated environments, although there was a large variation in the ratio viable spores/total spore count from 0.001 to 0.62 (review by Eduard & Heederik, 1998).

6.3. Conclusions

Exposure levels in highly contaminated environments often exceed 10^4 cfu/m^3 and 10^6 spores/m^3 and may even exceed 10^8 spores/m^3. In indoor environments without fungal contamination, fungal levels are generally below 10^3 cfu/m^3 and lower than in outdoor air. Fungal levels in buildings with fungal growth can be somewhat higher than in buildings without such contamination. Although exposure levels in common indoor environments are not exactly known, these levels are probably several orders of magnitude lower than in highly contaminated environments.

7. Uptake, distribution, and elimination

7.1. Uptake

The main exposure route of fungal spores is by inhalation. The size, shape, and surface structure of the individual spores as well as aggregates are of major importance for their aerodynamic behaviour and thus the region where inhaled spores deposit in the airways. Individual spores of fungi are typically 2–10 µm in size. Spores of actinomycetes are smaller, typically 0.5–1.5 µm. Due to the variable spore size of single spores, the presence of aggregates, differences in density, and absorption of water vapour by inhaled spores, fungal spores may be expected to deposit in the whole respiratory system. A substantial fraction of the smaller spore types may reach the alveoli, whereas the larger spore types (>7–10 µm) do not. Aggregates, which are larger, may deposit to a greater extent in the lower and upper airways rather than in the alveoli. For example, spores of the outdoor fungus *Alternaria alternata* are so large that they are not expected to reach the alveoli.

Gastrointestinal exposure may occur via mucociliary clearance of spores that have deposited in the airways and are swallowed when mucous reaches the throat.

Some pathogenic fungi may infect the skin, but such organisms are beyond the scope of this review.

7.2. Distribution

A limited number of in vivo studies have addressed dissemination of spores from the airways to other organs. All studies used *Aspergillus fumigatus* or *Aspergillus terreus.* In addition, a *Penicillium* species and *Rhizopus oryzae* were included in two of the studies, respectively. These studies are summarised below and described in more detail in Appendix 3 of Eduard (2007).

Two studies showed that spores of *A. fumigatus* disseminated to the spleen, liver, and kidneys in rabbits (Kurup, 1984) and mice (Waldorf et al., 1984). The latter study also showed that viable counts had cleared from these organs after 10 days. However, in a study by Schaffner et al. (1982), mice exposed to *A. fumigatus* spores showed no dissemination to

other organs. The latter study used more realistic conditions as airborne spores were inhaled and the dose may have been lower (4×10^3 spores/g body weight [bw]) than in the study by Waldorf et al. (1984) who exposed mice intranasally to a 20 times higher dose. However, Schaffner et al. (1982) estimated the dose after 2 hours by culture of lung tissue and probably underestimated the applied dose.

A study of intranasally instilled spores of *R. oryzae* in mice showed that spores migrated to the liver, spleen, and kidneys, and were still present in the liver and spleen 10 days after exposure (Waldorf et al., 1984). Two studies of *A. terreus* spores administered intratracheally to rats and rabbits showed only minor migration of spores to the tracheobronchial lymph nodes (Green et al., 1980; Olenchock et al., 1983).

In a rabbit study, Thurston et al. (1979) found a small fraction of spores in the digestive system 6 hours after aerosol exposure to *A. fumigatus* and a *Penicillium* sp. No fungi could be cultured a week after exposure. No other organs than the lung and the digestive system were studied.

In summary, these studies show different results even for the same species *A. fumigatus*. Dissemination to other organs than the lung and the digestive system was observed only for the facultative pathogenic organisms *A. fumigatus* and *R. oryzae*. However, only one nonpathogenic fungus, a *Penicillium* species, has been studied, and all studies showing dissemination to other organs applied intratracheal or intranasal instillation, which may have caused local overload of macrophages (Sections 10.1 and 12.1). One study found that inhaled spores of *A. fumigatus* and a *Penicillium* sp. reached the digestive system, suggestive of mucociliary clearance. However, the proportion of the inhaled dose was small and the fungi were cleared within a week, indicating that the exposure of the digestive system is limited.

7.3. Elimination

7.3.1. In vivo studies

Elimination of spores from lung tissue and phagocytosis and destruction of spores by pulmonary alveolar macrophages have been studied for several species of fungi and laboratory animals. The studies are summarised here (Table 8) and more details are given in Appendix 4 of Eduard (2007).

In three of the studies, animals were exposed to aerosols of *Aspergillus fumigatus* spores and the deposited dose was estimated by culture of lung tissue. Colony counts declined in Guinea pigs, rabbits, and immunised mice, although small numbers were detectable after 6 days or more (Voisin et al., 1971; Thurston et al., 1970; Schaffner et al., 1982). Counts of *Penicillium* sp. declined more slowly in rabbits inhaling spore aerosols, and colonies were still observed after 3 weeks (Thurston et al., 1979). *Saccharopolyspora rectivirgula* counts declined much slower in aerosol exposed Guinea pigs (Voisin et al., 1971). In a second study on *S. rectivirgula* in Guinea pigs, 10^2–10^3 times higher doses were applied by intratracheal instillation and a rapid decline in colony counts was found (Zaidi et al., 1971), similar to the results obtained for *A. fumigatus*. Elimination was delayed when exposure to *S. rectivirgula* spores was combined with hay dust, compared with exposure to spores alone (Zaidi et al., 1971). In two other studies using intratracheal instillation of *Candida albicans* cells in Guinea pigs and *Aspergillus terreus* spores in rats and rabbits, most microorganisms had been cleared after 1 day (Voisin et al., 1971; Olenchock et al., 1983). A study of mice intranasally exposed to *Rhizopus oryzae* showed culturable fungi in the lungs 10 days after exposure, although no quantitative data were presented (Waldorf *et al.,* 1984).

To sum up, although several studies found that the major part of culturable fungi was eliminated from the lung after a few days, elimination was not complete after a week, and could last considerable longer for *R. oryzae* and *S. rectivirgula*. The applied doses in the latter studies did not exceed the overload limit of 60 μm^3/macrophage suggested by Morrow (1992) (Section 8.1).

Spores of *A. fumigatus, A. terreus,* and *Penicillium chrysogenum* were rapidly phagocytised by alveolar macrophages in mice, rats, and rabbits (Green et al., 1980; Kurup, 1984; Waldorf et al., 1984; Cooley et al., 2000). Phagocytised spores of *A. fumigatus* and *P. chrysogenum* were killed by mouse alveolar macrophages (Waldorf et al., 1984; Cooley et al., 2000). However, spores of *A. fumigatus* seemed to resist rabbit alveolar macrophages in one study (Kurup, 1984). Mouse alveolar macrophages prevented germination of spores of *Rhizopus oryzae* but did not kill the spores (Waldorf et al., 1984). The role of alveolar macrophages in prevention of spore germination was demonstrated by mycelial growth of *S. rectivirgula* in the lungs of Guinea pigs that had been depleted of alveolar macrophages by treatment with antialveolar macrophage serum (Voisin et al., 1971). It was also observed that alveolar macrophages migrated from the peripheral alveoli to alveoli adjacent to the respiratory bronchioles (Green et al., 1980).

In conclusion, the alveolar macrophages are the primary line of defence against fungal spores. Alveolar macrophages killed spores from most tested species, and if spores withstood macrophage attack, germination was prevented.

7.3.2. In vitro studies

In vitro studies have been carried out with viable and killed spores of various microbial species using different cell types, and a variety of responses were studied. The studies are described in more detail in Appendix 5 of Eduard (2007).

Spores from *Aspergillus fumigatus, Aspergillus candidus,* and *Penicillium ochrochloron* attached readily to different phagocytic cells. *P. ochrochloron* spores were more easily phagocytised than *A. fumigatus* spores, and heat-killed *A. fumigatus* spores were phagocytised more rapidly than inert particles (Robertson et al., 1987; Nessa et al., 1997a, 1997b). Ingested spores of *Aspergillus flavus* and *Aspergillus niger,* but not *A. fumigatus,* were killed by rabbit alveolar macrophages and the latter even germinated after 4 hours (Kurup, 1984).

Killing of microorganisms by phagocytes involves reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO). ROS production by different phagocytic cell types differed between

Table 8. Elimination of spores of fungi and actinomycetes from the lung in animal studies.

	Animals		Exposure			Observation time	Elimination		Reference
Microorganism	Species	No./ group[a]	Route	Dose as specified in the study	Dose estimated as spores/g bw[b]		From lung tissue (% of initial dose)	Mechanistic observations	
Fungi									
Aspergillus fumigatus									
	Mouse, immunised	9	ae	3×10^5 cfu/g lung after 2 h	4×10^3	6 d	2 d: <1% 6 d: +		Schaffner et al. (1982)
	Guinea pig	100	ae	7×10^6 cfu after 2 h	4×10^4	12 d	1 d: 10% 4 d: 0.1% 10 d: +		Voisin et al. (1971)
	Rabbit	6	ae	5×10^5–4×10^6 cfu/g lung after 1 h	1×10^4–8×10^4	3 wk	1 d: 1–10% 2 d: 0.1–2% 1.5–3 wk: nd		Thurston et al. (1979)
	Mouse	10	i.n.	1×10^6 spores	4×10^4	10 d		18 h: 70% of spores in AM were killed; 10 d: no germination.	Waldorf et al. (1984)
	Rabbit	5	i.t.	1×10^7 spores	4×10^3	4 h		1 h: 53% of observed spores in AM; 4 h: 22%.	Kurup (1984)
Aspergillus terreus									
	Rabbit	Not given	i.t.	7×10^6 spores	4×10^3	2 d		Rapid uptake by AM, complete after 3 h.	Green et al. (1980)
	Rat		i.t.	5×10^7 spores	1×10^5			Very few PMN observed.	
	Rat	2	i.t.	5×10^7 spores	1×10^5	24 h	3 h: 100%	0 h: 48% of observed spores in AM; 24 h: 98% in rats.	Olenchock et al. (1983)
	Rabbit	2	i.t.	7×10^6 spores	4×10^3		24 h: 20%	Qualitatively similar results in rabbits.	
Candida albicans									
	Guinea pig	20	i.t.	1×10^7–12×10^7 cells	3×10^4–4×10^5 cells	5 d	3 h: 100% 8 h: 3% 24 h: 0.1%		Voisin et al. (1971)
Penicillium chrysogenum									
	Mouse	Not given	i.n.	1×10^4 spores	4×10^2	24 h		3-24 h: Spores were phagocytised and digested by AM.	Cooley et al. (2000)
Penicillium sp.									
	Rabbit	3	ae	1×10^5–5×10^5 cfu/g lung after 1 h	2×10^3–1×10^4	3 wk	1 d: 7–30% 2 d: 4–7% 3 wk: 0.02–0.03%		Thurston et al. (1979)
Rhizopus oryzae									
	Mouse	10	i.n.	1×10^6 spores	4×10^4	10 d		18 h: 20% of spores in AM were killed; 10 d: no germination.	Waldorf et al. (1984)
Actinomycetes									
Saccharopolyspora rectivirgula									
	Guinea pig	90	ae	1×10^6–8×10^6 cfu after 2 h	3×10^3–3×10^4	2 mo	2–3 wk: 10% 2 m: +	Mycelial growth in anti-AM serum–treated animals.	Voisin et al. (1971)
	Guinea pig	30	i.t.	800 µg spores	4×10^6	7 d	1 d: 10% 3 d: 0.1% 7 d: +		Zaidi et al. (1971)
	Guinea pig	30	i.t.	800 µg spores + 75 mg hay dust	4×10^6	9 d	5 d: 100% 9 d: 10%		Zaidi et al. (1971)

[a]Number of animals in exposed and control group.

[b]Dose estimated as described in Section 10.1.

+: a few colony counts were observed; ae: aerosol; AM: alveolar macrophages; cfu: colony-forming units; i.n.: intranasal; i.t.: intratracheal; nd: not detectable; PMN: polymorphonuclear leukocytes.

fungal and actinomycete species (Shahan et al., 1994, 2000; Ruotsalainen et al., 1995; Hirvonen et al., 1997a). Differences were also observed between isolates of the same species and even the same strain grown on different substrates when tested with a mouse macrophage cell line (Ruotsalainen et al., 1998; Hirvonen et al., 2001). Similar differences were also observed for NO and inducible NO synthase production (Hirvonen et al., 1997a, 1997b, 1997c; Ruotsalainen et al., 1998; Jussila et al., 1999; Murtoniemi et al., 2001, 2002; Huttunen et al., 2003, 2004; Penttinen et al., 2005).

The production of cytokines as interleukin (IL)-6, IL-1, IL-1β, and tumour necrosis factor alpha (TNFα) by phagocytic cells, and cytotoxicity in a mouse macrophage cell line varied between species, cell types, and the medium used for culture of the microorganisms, including building materials (Nessa et al., 1997b; Ruotsalainen et al., 1998; Shahan et al., 1998; Huttunen et al., 2003; Pylkkänen et al., 2004). Cytotoxic strains of *Stachybotrys* produced satratoxin but did not induce IL-6 and TNFα in a mouse macrophage cell line whereas nontoxic strains induced these inflammatory mediators (Nielsen et al., 2002), indicating that mycotoxins impair the cytokine response.

Positive interactions were found between S*tachybotrys chartarum* with the actinomycete *Streptomyces californicus* on IL-6 production, apoptosis, cell-cycle arrest, and caspase-3 enzyme activity in a mouse macrophage cell line, whereas no or weaker responses were found for NO and TNFα production (Huttunen et al., 2004; Penttinen et al., 2005). Two other fungi did not show such interactions with this actinomycete.

Spore viability has shown different effects on the elicited inflammatory responses of macrophages to spores. The response to viable and dead spores was similar regarding superoxide anion induction, NO production, and phagocytosis. However, killed spores induced less ROS than viable spores, whereas both higher and lower cytotoxicity was observed in a mouse macrophage cell line. These studies included many fungal and actinomycetes species (Shahan et al., 1994; Hirvonen et al., 1997a). Viable *A. fumigatus* induced production of a variety of cytokines whereas killed spores did not (Pykkänen et al., 2004). However, Shahan et al. (1994) found no difference in macrophage superoxide production between viable and killed spores in a similar study of this fungus. A recent study by Hohl et al. (2005) showed a large increase in TNFα and macrophage inflammatory protein (MIP)-2 production by murine alveolar macrophages after exposure to alive *A. fumigatus* spores, whereas no response was observed after exposure to heat-killed spores. However, when spores were incubated, the inflammatory response of heat-killed spores increased with incubation time, indicating that germination of spores alters their inflammatory potential.

7.3.3. Discussion

The in vivo studies demonstrated that most fungal spores were eliminated from the lung within a few days, although elimination was not complete and could last considerably longer for *Rhizopus oryzae* and *Saccharopolyspora rectivirgula*. It was also observed that alveolar macrophages phagocytised the spores, and although some spores maintained viability, they did not germinate. One study demonstrated that the alveolar macrophages were crucial in germination prevention.

The exposure levels used in in vivo studies were rather high. The lowest dose was 2×10^3 spores/g bw, which corresponds to a human dose of $>10^8$ spores. Such doses can be inhaled during an 8-hour workday at exposure levels $>10^7$ spores/m^3, i.e., at the higher end of occupational exposures and many orders of magnitude higher than in environments with common indoor exposure to fungal spores (Tables 5–7). However, the dose was estimated by culture of lung tissue 2 hours after aerosol exposure and the actual dose may have been substantially higher (Section 10.1). Thus, the delayed elimination of spores of some species may not be relevant for populations working in highly contaminated environments except in extreme exposure situations.

In vitro studies further documented that alveolar macrophages bind spores rapidly to their cell walls. The alveolar macrophages phagocytise attached spores, kill ingested spores by NO and ROS, and may produce cytokines. Differences in induced macrophage NO and ROS production were observed among fungal species, isolates of the same species, and even isolates grown on different substrates, but results were not consistent across studies. Similarly, results on production of cytokines such as TNFα and IL-6 were not consistent, although these and other cytokines were demonstrated in response to spores from many species. The use of different cell types in in vitro tests, together with the presence of secondary metabolites in the spore preparations, may account for some of the differences.

Some in vitro studies showed that spores of fungi and actinomycetes were able to resist and even kill alveolar macrophages, although results were not consistent.

The decline of culturable microorganisms in lung tissue may also be due to migration of spores to other organs. This was shown for the opportunistic pathogens *A. fumigatus* and *R. oryzae* that seem able to resist pulmonary defence mechanisms.

Some results are indicative of mucociliary clearance of spores in the respiratory tract. Alveolar macrophages with phagocytised spores migrated from the peripheral alveoli to those closest to the terminal bronchioles. The detection of microorganisms in the digestive system indicates that they probably had been swallowed.

The effect of viability was explored in in vitro studies and one in vivo study. Although results were variable, there are indications that the elicited response from viable spores can be stronger than that from dead spores when they are able to germinate.

8. Mechanism of toxicity

Viable microorganisms such as spores of fungi and actinomycetes are different from chemical agents, as they may

be able to germinate and proliferate in the host. When the host's defence systems fail, toxicity may arise, leading to various clinical outcomes such as organic dust toxic syndrome (ODTS), mucosal membrane irritation, and chronic restrictive and obstructive lung diseases such as hypersensitivity pneumonitis and asthma.

8.1. The innate and adaptive response to fungi

The alveolar macrophages are the first line of defence against microorganisms at the alveolar level. Their role in elimination of fungal spores has been described in Section 7.3. Alveolar macrophages phagocytise spores and migrate to the airways where they are removed by mucociliary clearance. Morrow (1992) reviewed studies on migration of rat alveolar macrophages from the alveoli into the airways. The migration was impaired when the total volume of phagocytised nonsoluble particles exceeded approximately 60 μm^3 per alveolar macrophage. If this clearance limit also applies to fungal spores, it corresponds to human alveolar doses of 6×10^9 to 9×10^{10} of spores with sizes 5 and 2 µm, respectively, as the human lung contains approximately 6×10^9 alveolar macrophages (Stone et al., 1992). This relates to exposure levels of 10^9 to 2×10^{10} spores/m^3 during a work shift assuming an alveolar ventilation of 10 L/min for 8 hours. Such levels are rare in the work environment (Section 6.1) but have been reported in relation to fever attacks typical of ODTS (Sections 8.3 and 11.4.2). It is therefore possible that clearance overload leading to prolonged residence time of fungal spores at the alveolar level is a contributing factor to ODTS.

The defence of human and murine macrophages against hyphae is not effective, which has been shown for the pathogenic species *Aspergillus fumigatus* and *Histoplasma capsulatum* (KImberlin et al., 1981; Schaffner et al., 1982). However, human neutrophils destroyed the mycelial form of *Aspergillus fumigatus* and *Rhizopus oryzae* by attaching to the hyphae and by extracellular production of ROS (Diamond et al., 1978; Schaffner et al., 1982).

Several receptors have been found on human and murine phagocytic cells, i.e., the alveolar macrophages, neutrophils, and dendritic cells, by which these cells attach to inhaled microorganisms. The Toll-like receptors (TLRs) are probably the most important receptors for microbial recognition. It has been shown that TLR2, TLR4, TLR6, and TLR9 on mouse and human phagocytes bind to the pathogenic fungi *Aspergillus fumigatus, Cryptococcus neoformans,* and *Candida albicans* (Bellocchio et al., 2004a; review by Netea et al., 2004). The binding of *A. fumigatus* to TLR2 and TLR4 promoted fungicidal activity in neutrophils through different oxidative pathways, whereas TLR3, TLR5, TLR6, TLR7, TLR8, and TLR9 further modulated the response (Bellocchio *et al.,* 2004b). Other innate receptors involved in fungal recognition are the IL-1 receptor that is structurally related to the TLRs, the mannose receptor, and the Dectin-1 receptor that binds to β-glucans (Bozza et al., 2002; Bellocchio et al., 2004a; Netea et al., 2006). These receptors seem to act in combination in the recognition of pathogenic microorganisms. For example, TLR2, TLR4, the Dectin-1 receptor, and the mannose receptor have been shown to bind to different molecules on the cell wall of *Candida albicans* yeast cells (Netea et al., 2006).

Fungal spores and hyphae bind differently to the innate pattern recognition receptors. Conidia of *Aspergillus fumigatus* were recognised by TLR2, TLR4, and TLR9 on human and murine neutrophils, whereas hyphae were only recognised by TLR4 (Bellocchio et al., 2004b). Gantner et al. (2005) found that yeast cells of *Candida albicans* bound to the Dectin-1 receptor on murine macrophages, which the authors ascribed to β-glucan being exposed on the outer cell wall at the scars formed by the budding yeast cells. Hyphae were not recognised probably because the β-glucan in the hyphal cell wall was covered by other compounds. The Dectin-1 receptors on murine and human macrophages were also found to bind preferentially to germinating spores of *A. fumigatus* compared to resting spores. This was similarly explained by disruption of the layer that covers the β-glucan on the spore wall during spore germination (Hohl et al., 2005; Gersuk et al., 2006). Thus, the composition of the cell wall surface has a major effect on the binding to phagocytic cells.

Other pattern recognition receptors are present on surfactant proteins A and D that are produced by epithelial cells in the alveoli and bronchi. These proteins play an important role in the lung by reducing the surface tension on the alveoli, but they also contain a mannose receptor that binds to a range of sugars that can be present on invading microorganisms including fungi; review by Turner (2003). After binding, the surfactant proteins can activate complement directly, or they can stimulate phagocytosis by binding to collectin receptors on phagocytic cells; review by Crouch (2000).

Recent publications have addressed the role that antigen-presenting pulmonary dendritic cells play in the immune responses to fungi. Dendritic cells phagocytise pathogens and present their antigens to precursor T-helper (Th) cells, which may mature into Th1 and Th2 cells. The Th1 cells mediate a cellular response by stimulating macrophages to phagocytise the pathogen and T cells to develop into cytotoxic T cells that phagocytise and kill specific organisms. The Th2 cells stimulate B cells to proliferate into specific antibody-producing plasma cells. The Th1 and Th2 cells further modulate the type of antibodies that are produced by secretion of different cytokines. Th1 cells promote the development of IgG antibodies that bind specifically to antigens and facilitate their uptake by phagocytic cells. Th2 cells may stimulate B cells to produce IgE antibodies and can also induce proliferation of mast cells and eosinophilic leukocytes, which are important characteristics of the allergic response. The balance between Th1 and Th2 cells is thus important for the type of immune response that is mounted against invading organisms. Recently, T regulatory cells involved in suppression of the Th2 response and thought to prevent sensitisation and allergic disease were described; review by Robinson et al. (2004). However, their role in fungal allergy is not clear.

Mouse dendritic cells have been shown to respond differently to spores and hyphae (Bozza et al., 2002). Resting spores of *Aspergillus fumigatus* were more efficiently phagocytised than swollen conidia and hyphae in vitro. In vivo mouse alveolar dendritic cells with phagocytised spores translocated through the alveolar epithelial barrier migrated to the thoracic lymph nodes and the spleen and underwent functional maturation. Dendritic cells produced IL-12p70 and TNFα after ingestion of spores, which primed the maturation of precursor Th cells to interferon gamma (IFNγ)-producing Th1 cells, whereas ingestion of hyphae induced IL-4, IL-10, and TNFα production and priming of IL-4–producing Th2 cells (Figure 3).

Similar findings were reported in an in vitro study of *Candida albicans*. Dendritic cells that had ingested hyphae primed Th2 cells in mice, whereas dendritic cells primed Th1 cells after ingestion of yeast cells (d'Ostiani et al., 2000). A difference from *Aspergillus fumigatus* was, however, that hyphae of *Candida albicans* escaped from the phagosomes, whereas yeast cells were destroyed. The response of dendritic cells to fungi therefore seems to depend both on the species and on the cellular form of the organism. The production of fungal allergens during germination and mycelial growth is likely to play a role (Section 3.6). It seems therefore possible that viable spores may induce an allergic response if they germinate in the airways. Some support for this hypothesis is found in in vivo studies of viable and/or germinating spores (Sections 10.3, 10.4.2, and 10.5).

Also epithelial cells seem to be involved in interactions with fungi. It has been proposed that proteases present in fungi and other allergens may facilitate passage of allergens through the epithelial barrier; review by Kauffman and van der Heide (2003).

Mycotoxins may further modulate the immune response, as many mycotoxins are cytotoxic to immune cells. The mycotoxins citrinin, gliotoxin, and patulin induced a stronger suppression of human Th1 cells than Th2 cells in vitro, which favours an allergic response to the toxin-producing fungi (Wichmann et al., 2002). Mycotoxins can also have other health effects (Section 3.6).

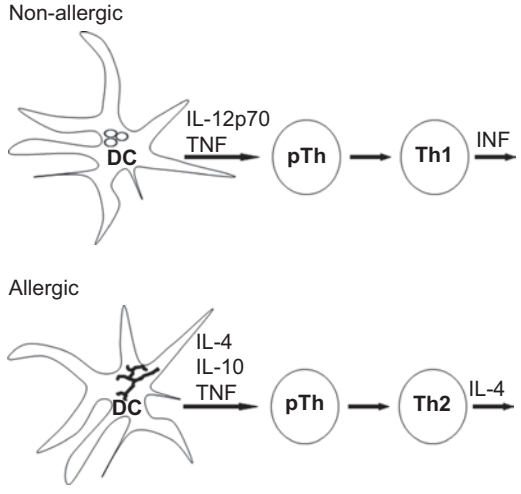

Figure 3. Differential maturation of precursor helper T cells (pTh) by dendritic cells (DC). A nonallergic response with development of Th1 cells was observed after DCs ingested spores, whereas ingestion of hyphae induced an allergic response with development of Th2 cells. Based on Bozza et al. (2002).

8.2. Allergic responses

Allergy has been defined in different ways. In this document the definitions of allergy and hypersensitivity pneumonitis given by the European Academy of Allergology and Clinical Immunology are used (Johansson et al., 2001; see "Terms as used in this document").

IgE-mediated allergy is probably the most important allergic mechanism in the general population. Exposure of sensitised individuals (i.e., with elevated IgE antibodies) to the corresponding allergen causes mast cells to release histamine and other inflammatory mediators. In asthmatic patients, histamine release causes constriction of the bronchi by smooth muscle contraction, and cytokines attract eosinophils. The subsequent eosinophilic inflammation further constricts the airways by swelling of the mucosa, resulting in airway obstruction that can be life-threatening. In patients with allergic rhinoconjunctivitis, the mast cells produce the same inflammatory agents, resulting in sneezing, running or blocked nose, and eye irritation. Although not all individuals with detectable IgE are symptomatic, the presence of specific IgE antibodies is a predictor of future allergic disease if exposure to the allergen continues (review by Platts-Mills, 2001).

Few occupational studies of IgE sensitisation to fungi have been published. These studies provide conflicting information on the occurrence of sensitisation and IgE-mediated diseases in working populations exposed to fungi. This may be due to the limited number of available assays for detection of IgE to fungal allergens in serum and the low sensitivity of these assays (Section 11.2.2).

Individuals with IgE against commonly occurring allergens also have greater risk of developing IgE against occupational allergens, for example, fungal amylase in bakers (Houba et al., 1998) and rodent allergens in laboratory animal workers (heederik et al., 1999). Such individuals are called atopic and their identification is important as they may represent a sensitive group. Although fungal allergy is quite common in atopic individuals of the general population, the role of atopy for fungal allergy in occupational populations is unclear.

Another type of allergic disease that seems to be more important in populations occupationally exposed to fungi is hypersensitivity pneumonitis, which is also called allergic alveolitis. This disease is characterised by alveolar and bronchiolar inflammation, and is caused by inhalation of spores from fungi and actinomycetes, and also by other allergens. Typical symptoms and clinical findings are attacks with fever, chills, dry cough, dyspnoea, basal crepitations, nodular bilateral X-ray changes, malaise and headache, and declines in forced vital capacity (FVC) and gas diffusion capacity. IgG but not IgE can usually be detected. The attacks are similar to

those of ODTS (Section 8.3), but patients with ODTS recover within a few days without persisting symptoms or clinical findings. Acute attacks of hypersensitivity pneumonitis occur a few hours after episodes with massive exposure to spores of fungi and/or actinomycetes. Recurrent attacks may eventually progress into pulmonary fibrosis if exposure continues, and may even be fatal (Rose, 1996; Patel, 2001). Patients typically work in environments where high exposures occur repeatedly.

Hypersensitivity pneumonitis is thought to be an allergic disease, although the mechanisms are not entirely clear. The disease is not IgE mediated, as IgE antibodies are not generally found in patients with hypersensitivity pneumonitis. Specific IgG antibodies to fungi can often be demonstrated in serum of these patients, indicating a type III allergic reaction. However, the role of IgG antibodies in the disease is unclear, as these antibodies are common in healthy exposed individuals. The presence of serum IgG antibodies is therefore regarded as a marker of exposure and not of disease (Burrell & Rylander, 1981; Section 9.1). Additionally, type IV hypersensitivity is suggested by the presence of granuloma in the lungs of the patients (Section 11.5.1.), and by granuloma formation in animal studies (Sections 10.4 and 10.5), indicating the involvement of cytotoxic T cells. Host factors may play a role as well because only a small proportion of the exposed individuals develop the disease (Bourke et al., 2001; Section 11). Neutrophilic inflammation is generally observed in this disease and production of the pyrogenic cytokines TNFα and IL-6 by neutrophils probably explain the fever attacks that are typical for hypersensitivity pneumonitis.

Although the mechanisms of hypersensitivity pneumonitis are not entirely clear, the allergic nature of the disease is clearly demonstrated by bronchial challenge to fungal extracts and even spores in numerous case studies and a few epidemiological studies (Sections 11.2.1 and 11.5.1.).

8.3. Nonallergic responses

Chronic bronchitis and ODTS are generally regarded as nonallergic diseases (von Essen et al., 1990; Parkes, 1994). ODTS is also described as toxic alveolitis or inhalation fever (Rask-Andersen, 1989; von Essen, 1990). ODTS patients develop febrile attacks with chills, headache, malaise, cough, and dyspnoea 4–8 hours after massive exposure to some substances, e.g., metal fumes, organic particles, and spores from fungi and actinomycetes. IgG antibodies against fungi are often not detectable. Patients recover within a few days without functional and X-ray changes or persisting symptoms, which distinguishes this condition from hypersensitivity pneumonitis.

Mucous membrane irritation may be due to allergic rhinoconjunctivitis, but is prevalent also in individuals without atopy. A substantial proportion of the patients with diagnosed asthma and rhinoconjunctivitis do not have atopy or eosinophilic inflammation but probably neutrophilic inflammation (Pearce et al., 1999; Douwes et al., 2002; Zacharasiewicz et al., 2003. Nonallergic diseases are therefore important outcomes in populations exposed to fungi.

Most experimental and animal studies report nonallergic inflammation after challenge to fungal spores. The following mechanisms are supported by these studies (reviewed in Section 7). Alveolar macrophages phagocytise spores and destroy spores in phagolysosomes by ROS and by non–oxygen-dependent mechanisms involving lysosomal enzymes. Spores from some species, and hyphae in general, are resistant to destruction by alveolar macrophages. The alveolar macrophages can produce a variety of cytokines, among which IL-8 is most important to attract neutrophils. The neutrophils can phagocytise and destroy hyphae and swollen spores by oxidative and nonoxidative mechanisms. The fungicidal agents are present in granules within the neutrophils and are released inside the cells, but they can also be excreted and cause tissue damage. Both activated alveolar macrophages and neutrophils can produce the pyrogenic cytokines TNFα and IL-6, which provides a likely explanation for febrile symptoms during attacks of ODTS.

8.4. Summary

The inflammatory response to spores is mainly nonallergic in occupational populations, although allergic diseases such as allergic asthma, allergic rhinoconjunctivitis, and hypersensitivity pneumonitis can be induced by exposure to fungi. The allergic and nonallergic responses indicate that different inflammatory mechanisms are involved, many of which are not fully elucidated. Fungi are recognised by phagocytic cells with innate receptors. They become phagocytised and induce both innate and adaptive immune responses. Hyphae and germinating spores are able to induce allergic responses, which may be due to allergen production during germination and growth.

Allergic asthma and rhinoconjunctivitis are IgE mediated, whereas hypersensitivity pneumonitis is most likely due to cellular hypersensitivity (type IV allergy) and/or IgG-mediated type III allergy.

Nonallergic asthma and rhinoconjunctivitis are at least as prevalent as the allergic forms of the diseases, and are probably mediated by neutrophilic inflammation. ODTS is characterised by similar acute symptoms as hypersensitivity pneumonitis, but the chronic effects seem to be absent. The fever attacks in both outcomes are probably caused by macrophage and neutrophilic inflammation. Macrophage overload may play a role in ODTS.

9. Biological monitoring

9.1. Markers of exposure

IgG antibodies may appear after repeated exposure to airborne fungal spores. This has been shown in several animal studies. An interesting study is the one by Thurston et al. who exposed rabbits once a day for 1–10 days to aerosols of *Aspergillus fumigatus* spores with doses ranging from 4×10^5 to 2×10^6 cfu/g lung tissue (Thurston et al., 1975). They found that precipitating antibodies only developed after exposure to the highest dose ($2 \cdot 10^4$ spores/g bw corresponding to a

human dose of more than 10^9 spores) and that exposure had to be repeated at least once. This is in agreement with the mechanism for immunisation through development of IgG antibodies (Roitt et al., 1996). After a single exposure, few or no animals developed IgG antibodies (Thurston et al., 1975, 1979; Olenchock et al., 1976; Kurup & Sheth, 1981; Olenchock et al., 1983; Nikulin et al., 1997), except in one study where rabbits were exposed to a very high dose of *A. fumigatus* (approximately 2×10^6 to 5×10^6 spores/g bw) (Morin et al., 1974). Cooley et al. (2000) exposed mice intranasally to doses of 1×10^4 spores of *Penicillium chrysogenum* once a week for 6 weeks. Both killed and viable spores caused decreased specific IgG_{2a} levels, although total IgG_{2a} levels increased after exposure to killed spores. The presence of specific IgG_{2a} antibodies before exposure indicates that the animals had been exposed to *P. chrysogenum* or other fungi cross-reacting with this species before the challenges, however. Thurston et al. (1975) found a relatively high threshold for precipitin formation against *A. fumigatus* in rabbits, which may explain why an increase in specific IgG_{2a} levels was not found in the study by Cooley *et al. (*2000).

Thus, specific serum IgG can be regarded as an indicator of fungal exposure. The relationship of these antibodies to disease has also been studied, but because healthy individuals also can have high IgG levels, they are not regarded as a marker of disease (Burrell & Rylander, 1981; Section 8.2). However, specific serum IgG is useful as an indicator of fungal exposure in the diagnosis of hypersensitivity pneumonitis where documentation of exposure is required (Richerson et al., 1989; Rose, 1996; Patel et al., 2001; Section 8.2).

Specific IgG antibodies to *Saccharopolyspora rectivirgula*, *Aspergillus, Penicillium,* and *Rhizopus* species in serum have been used as markers of exposure in studies of farmers and sawmill, cork, tobacco, and malt workers, but only two epidemiological studies were found that examined the relationship between serum IgG levels and fungal exposure levels. In wood trimmers, levels of serum IgG against *Rhizopus microsporus* and *Paecilomyces variotii* were associated with exposure levels of spores from these fungi in the preceding months (Eduard et al., 1992). Individual differences in the antibody response were large, however. In school children, levels of serum IgG against fungi from water-damaged schools correlated poorly with airborne levels of the fungi in the school building (Hyvärinen et al., 2003). This may be due to the relatively low exposure level in this environment, as the significance of specific IgG antibodies as marker of occupational exposure depends on the absence of other exposure routes, such as airborne exposure in the homes and outdoors and ingestion of contaminated food. The authors therefore did not recommend serum IgG levels as markers of exposure in schools.

Species from the same genera often cross-react, and species may even cross-react with less related species. However, IgG antibodies are fairly specific for fungi, although not at the species level.

Because the interindividual differences in the serum antibody response are large, the accuracy of individual serum IgG antibody levels as an exposure estimate is poor and the absence of IgG antibodies does not prove that a worker has not been exposed. Group mean IgG levels of similarly exposed individuals indicate exposure with better precision and have been used successfully as markers of exposure in epidemiological studies of populations working in highly contaminated environments, e.g., farmers and sawmill workers. Changes in intraindividual IgG levels indicate relative changes in exposure with better precision than a single individual measurement because the interindividual variability is eliminated; review by Eduard (1995).

To sum up, individual IgG antibody levels are difficult to interpret as exposure levels, and their use as markers of exposure has not been validated. However, the presence of IgG antibodies may indicate exposure to relatively high levels of specific fungi, which is valuable in the diagnosis of hypersensitivity pneumonitis.

9.2. Markers of effect

The demonstration of specific IgE antibodies to allergens is an important tool in the etiologic diagnosis of atopic disease. Allergens from *Cladosporium herbarum, Alternaria alternata,* and *Aspergillus fumigatus* have been best characterised and are used in commercial IgE tests (Kurup et al., 2000). The skin prick test and the radioallergosorbent test (RAST) in serum have been used predominantly but these methods are mainly semiquantitative. The allergenic composition of spores, mycelia, and culture fluid is different and fungal extracts also vary between strains. There is therefore a need for standardisation of fungal allergens (Salvaggio & Aukrust, 1981; Bush & Portnoy, 2001). Most allergen extracts have been prepared from culture fluid or mycelia. Positive tests thus indicate an allergic response to the fungus, but not necessarily to its spores since the allergen composition of the extract may deviate from that of spores. Another problem, which complicates the diagnosis of fungal allergy, is that allergic individuals can be sensitised to some but not all allergens from the same species and no allergen is common to all allergic individuals (Kurup et al., 2000). Major allergens have been recognised, however. For example, 85% of the patients allergic to *A. fumigatus* were positive when tested with the allergen Asp f 1 and 80% of the patients allergic to *Alternaria alternata* were positive to Alt a 1. However, only 50% of patients allergic to *C. herbarum* were positive to Cla h 1. Antigen extracts of this fungus used in diagnostic tests should therefore contain a combination of *Cladosporium* allergens. On the other hand, closely related (e.g., *Alternaria, Stemphylium,* and *Curvularia* species) and more distantly related fungi (e.g., *C. herbarum* and *A. alternata)* may contain or produce similar allergens. Allergic individuals may therefore cross-react to fungi they have not been exposed to (Hoffman, 1984; Larsen, 1994). RAST analyses can be carried out by the Pharmacia CAP system, which is an automated system that has been calibrated against the World Health Organization (WHO) IgE standard and allows quantitation of total and allergen-specific IgE. Recently an automated enzyme-linked immunosorbent assay system

has been developed, which may replace RAST for the detection of IgE in serum.

The RAST and the skin prick test may underestimate serum IgE against fungi. Griese et al. (1990) found a better sensitivity (90%) and specificity (86%) for a histamine-release test with fungal extracts compared with skin prick tests and RASTs in asthmatic children using bronchial provocation as the standard. Similar results have been reported by Nolte et al. (1990) but the sensitivity (67%) and specificity (80%) were lower. In one study, the presence of IgE antibodies against *Penicillium chrysogenum* was demonstrated by a histamine-release test in individuals who did not respond in skin prick tests and a fluorometric enzyme immunoassay (Magic Lite test) (Larsen et al., 1997). The histamine-release tests were performed by exposure of circulatory basophils that had been passively immunised with serum from the subjects to spores from fungi that had been isolated from the environment. The role of IgE in these tests was validated by testing sera from unexposed individuals and sera from subjects where IgE had been removed as negative controls. The authors claim that whole spores that can be used in histamine-release tests provide a better allergen source than allergens extracted from culture fluid, mycelium, or spores. This assumption seems reasonable since allergen extracts contain only soluble allergens, and proteolytic enzymes may have degraded allergens during purification. Furthermore, spores from fungi that are isolated from the environment can be used. However, the concentration of spores needed to induce histamine release seems very high (although the number of basophils was not given), 0.06–0.5 mg spores/ml, which is approximately equal to 1×10^7 to 5×10^7 spores/ml.

Sputum induction by inhalation of a hypertonic saline aerosol and nasal lavage have been used to study inflammatory markers and mediators in small-scale epidemiological studies of populations occupationally exposed to fungi (Section 11). Their usefulness as markers of fungal diseases is unclear at present.

10. Effects in animals

10.1. Dose considerations

Only animal studies in which the respiratory system was exposed to whole spores have been included. Exposure is either by intratracheal or intranasal instillation of a spore suspension, or by inhalation of an aerosol.

All dose estimates were recalculated to estimate the number of spores/g bw as a common unit since doses had been expressed in different ways. Viable counts of spore suspensions were assumed to be half of the total number of spores unless viability had been assessed. In case of aggregation, this is an underestimation. However, spore suspensions used for intratracheal or intranasal instillation were often homogenised, filtered, and inspected before use. Spore weights were computed from the mean of size ranges obtained from other references, as this information was seldom supplied in the studies. In addition, a globular shape and a specific gravity of 1 g/cm^3 were assumed. In aerosol inhalation studies, either airborne concentrations and exposure durations were given or viable counts in lung tissue obtained quickly (up to 4 hours) after the challenge. The latter method is sometimes used in instillation studies as well. Culture of lung tissue is likely to underestimate the applied dose substantially, as only 0.3% of an intratracheally instilled dose in rabbits could be cultured from lung tissue 4 hours after instillation (Kurup and Sheth 1981). Inhaled doses in animal studies were computed by multiplication of the airborne concentrations with the respiration rate of the animal (Calabrese, 1991). Finally, the applied doses were normalised by the number of spores/g bw using tables of body and lung weight (Melby & Altman, 1976).

Intratracheal and intranasal instillation circumvent the normal deposition processes in the airways, and distribute spores deeper into the lung. In rats, 4 times more cerium oxide with a mass median AED of 2.2 µm reached the lung after intratracheal instillation compared to inhalation (Pritchard et al., 1985). Further, intratracheally instilled dust was less homogeneously distributed in the lung compared with inhaled aerosols, with little dust reaching the periphery. This may lead to local overload conditions even if the average dose does not exceed the overload limit suggested by Morrow (1992) (Section 8.1). Pritchard et al. (1985) recommended not to extrapolate dose-response relationships from animal studies that applied intratracheal instillation to the human condition.

The tracheobronchial structure and dimensions of the human lung differ from the lungs of laboratory animals, especially small rodents. Based on different deposition models in the airways and dose metrics, Jarabek et al. estimated that the exposure level applied in an animal experiment has to be multiplied by a factor of 0.03–0.2 for particle number per alveolus, and by 1–7 for particle mass per unit area for particle sizes ranging from 0.3 to 6 µm (Jarabek et al., 2005). Finally, the degree of aggregation of fungal spores is often unknown, a problem that applies to human as well as animal studies.

Macrophage overload may occur when the alveolar dose of spores 2 and 5 µm in size exceeds approximately 10^6 and 10^5 spores/g bw in humans, respectively. These limits are similar in rats that have approximately 2.8×10^7 alveolar macrophages, or 9×10^4 per g bw assuming a body weight of 300 g, whereas humans have approximately 6×10^9 alveolar macrophages, or $1 \cdot 10^5$ per g bw assuming a body weight of 60 kg (Morrow, 1992; Stone et al., 1992; Section 8.1).

Comparisons between studies can be difficult because of methodological differences. Comparisons within studies are therefore more reliable and are given most weight.

A neglected issue in studies using instillation of spore suspensions is that spores may be metabolically activated. Green et al. (2003) showed that germinating spores had higher allergen content than resting spores, and Hohl et al. (2005) and Gersuk et al. (2006) found that the composition of the surface antigens on the cell wall of *Aspergillus fumigatus* spores changed during germination. It is therefore possible that the responses in studies using intranasal or

intratracheal instillation of spore suspensions differ from results from aerosol exposure to dry spores.

10.2. Irritation

No studies of irritation have been found.

10.3. Sensitisation

Only two experimental studies of sensitisation with whole spores were found. Hogaboam et al. (2000) exposed non-sensitised mice, and mice sensitised by injections with *Aspergillus fumigatus* antigen, intratracheally to a single dose of 5×10^6 spores (2×10^5 spores/g bw) of *A. fumigatus.* Both groups developed specific serum IgE antibodies after 3 days that lasted at least to the end of the observation period of 30 days. The IgE level in the sensitised group was 5 times higher. Cooley et al. (2000) exposed mice intranasally to 1×10^4 spores (7×10^2 spores/g bw) of *Penicillium chrysogenum* once a week for 6 weeks. After exposure to killed spores, specific serum IgE levels decreased whereas specific serum IgE and IgG_1 levels increased after exposure to a spore preparation containing 25% viable spores.

The two studies indicate that viable spores can induce serum IgE. It should be noted, however, that both studies applied spore suspensions, which may have activated the spores and induced allergen production. Exposure to non-viable spores did not induce IgE antibodies, and IgE levels even decreased, indicating that the animals had developed some degree of sensitisation before the experiment. This was not mentioned in the paper. Sensitisation might have occurred from ambient exposure, as *P. chrysogenum* is prevalent in common indoor air although the animals were kept in a high-efficiency particulate air (HEPA)-filtered room. The induction of IgG_1 antibodies in mice after exposure to viable spores in the latter study also indicates the involvement of Th2 cells and an allergic response.

10.4. Effects of single exposure

Effects of single exposures other than irritation and sensitisation have been studied in 11 species of fungi and 3 species of actinomycetes in rabbits, rats, mice, and Guinea pigs. The studies are summarised in Table 9.

10.4.1. Mortality

Mortality was observed after a single exposure to toxic and nontoxic *Stachybotrys chartarum* in rat pups, with lethal doses for 50% (LD_{50}) and 18% (LD_{18}) of the animals of 3×10^5 spores/g bw and 8×10^5 spores/g bw, respectively (Yike et al., 2002). For *Aspergillus fumigatus,* an LD_{11} of 6×10^5 spores/g bw was found in adult mice (Schaffner et al., 1982), and mortality in rats varied from 0/3 to 3/3 after exposure to 6×10^6 spores/g bw of five different strains (Land et al., 1989). The *A. fumigatus* strains from the latter study were tested for production of the mycotoxin gliotoxin but none produced the toxin. Although the presence of mycotoxins in the spores is likely to be an important factor for mortality, the toxicity of these fungi was not solely dependent on mycotoxins. In one study of *A. fumigatus,* the dose was quantified by culture of lung tissue and substantially underestimated (Schaffner et al., 1982). At the applied doses, overload of alveolar macrophages is very likely, which may have contributed to the observed mortality.

10.4.2. Inflammatory markers

Bronchoalveolar lavage. Neutrophils and/or alveolar macrophages increased in rats, mice, and Guinea pigs after exposure to *Aspergillus fumigatus, Aspergillus versicolor*, *Penicillium spinulosum,* toxic *Stachybotrys chartarum, Saccharopolyspora rectivirgula,* and *Streptomyces californicus* (Fogelmark et al., 1991; Rao et al., 2000a; Hogaboam et al., 2000; Jussila et al., 2002, 2003a, 2003b; Yike et al., 2002) and in sensitised mice also after exposure to *Cladosporium herbarum* (Havaux et al., 2005). Lymphocyte counts were increased after exposure to toxic *S. chartarum* and exposure of sensitised animals to *A. fumigatus* (Rao et al., 2000a; Hogaboam et al., 2000; Yike et al., 2002). Alveolar macrophage counts decreased after exposure of mice to the highest dose of *P. spinulosum* (Jussila et al., 2002a). Eosinophil counts were increased after exposure of mice to *Alternaria alternata* (Havaux et al., 2005), Guinea pigs to *S. rectivirgula* (Fogelmark et al., 1991) and rats to toxic *S. chartarum* (Rao et al., 2000a). In sensitised mice, eosinophil responses were observed to *A. fumigatus* (Hogaboam et al., 2000), *A. alternata* and *C. herbarum* and in nonsensitised mice also to *A. alternata* (Havaux et al., 2005).

Many studies showed transient increases in lung cell counts after exposure to relatively high doses of 2×10^5 spores/g bw or more. However, one study showed that alveolar macrophage counts decreased after exposure to a very high dose of 4×10^6 *P. spinulosum* spores/g bw *(*Jussila et al., 2002a). Comparisons within studies showed a stronger response to toxic *S. chartarum* compared to a nontoxic strain of the fungus (Rao et al., 2000b; Yike et al., 2002), and to *A. alternata* compared to *C. herbarum* (Havaux et al., 2005). Dose-related responses were observed for *A. versicolor* (Jussila et al., 2002b) and toxic *S. chartarum* spores (Rao et al., 2000b). The responses in alveolar macrophage, neutrophil, eosinophil, and lymphocyte counts were stronger in sensitised animals than in nonsensitised animals (Hogaboam et al., 2000; Havaux et al., 2005). Eosinophil counts were induced by *A. alternata, C. herbarum* (in sensitised animals), toxic *S. chartarum,* and *S. rectivirgula* (Fogelmark et al., 1991; Hogaboam et al., 2000; Havaux et al., 2005). In one of the studies, four fungal species, including *A. fumigatus,* did not induce eosinophils or any other cells by doses up to 2×10^5 spores/g bw. Spores were applied by inhalation, which reduces the dose that reached the alveolar region compared to intratracheal or intranasal instillation applied in the other studies (Fogelmark et al., 1991).

Increases in the number of inflammatory cells were parallelled by increases in the cytokines TNFα, IL-1β, and IL-6 and markers of tissue damage, i.e., lactate dehydrogenase, albumin, and haemoglobin in rats (Rao et al., 2000a, 2000b; Yike et al., 2002), in mice (Jussila et al., 2001, 2002b) and in specific pathogen–free mice (Jussila et al., 2002a,

Table 9. Animal experiments with single exposures to spores from fungi and actinomycetes.

	Animals			Exposure					
Microorganism	Species	Specific pathogen free	No./ group[a]	Route	Dose as specified in the study	Dose estimated as spores/g bw[b]	Observation time	Effects	Reference
Alternaria alternata									
	Mouse	No	5	i.n.	2×10^5 spores	1×10^3	24 h	*Nonsensitised animals*. BAL: Increased eosinophils. No changes in cytokines. *Sensitised animals*. BAL: Increased AM, lymphocytes, neutrophils, and eosinophils. Increased IL-4, IL-5, and IL-13.	Havaux et al. (2005)
Aspergillus fumigatus									
	Guinea pig	No	Not specified	ae	7×10^5–3×10^7 spores/m^3, 4 h	6×10^1–3×10^3	4 h	BAL: No changes in AM, lymphocytes, neutrophils and eosinophils. Lung histology: No changes.	Fogelmark et al. (1991)
	Rabbit	No	4	ae	4×10^4–3×10^5 cfu/g lung	4×10^2–3×10^3	24 h	Blood: Decrease in PaO_2; dose-related complement decrease.	Olenchock et al. (1976)
	Rabbit	No	3	ae	2×10^6 cfu/g lung	2×10^4	3 wk	Lung histology: Transient granuloma formation with spores, lymphocytes and heterophils; eosinophils present in granulomas with germinated spores.	Thurston et al. (1979)
	Mouse	No	5–18	ae	2×10^5–8×10^6 cfu/animal	2×10^4–6×10^5	28 d	Mortality: 2/18 animals died 21 d after the highest applied dose.	Schaffner et al. (1982)
	Rabbit	No	2	i.t.	1×10^7 spores/ animal	4×10^3	21 d	Lung histology: Transient granulomas with fungi, and central necrosis (7–14 d).	Kurup & Sheth (1981)
	Mouse	Yes	4–5	i.t.	5×10^6 spores/ animal	2×10^5	30 d	*Nonsensitised animals*. Lung tissue: Transient increase in MCP-1/CCL2. No changes in MCP-3/CCL7 and MCP-5/CCL12. *Sensitised animals*. Lung tissue: Increase in MCP-1/CCL2.	Blease et al. (2001)
Aspergillus fumigatus									
	Mouse	Yes	5	i.t.	5×10^6 spores/ animal	2×10^5	30 d	*Nonsensitised animals*. Blood: Increased serum IgE. BAL: Increase in macrophages; transient increases in neutrophils. Lung tissue: Increased IL-18; transient increases in IL-4, IL-13, eosinophils, and lymphocytes; transient decrease in IL-10. Lung function: Transient increases in bronchial hyperreactivity. *Sensitised animals*. Stronger increase in serum IgE BAL: Similar increases in macrophages and transient increases in neutrophils. Lung tissue: Stronger increase in IL-18; stronger transient increases in IL-4, eosinophils, and lymphocytes; similar transient increase in IL-13 and transient decrease in IL-10 (all effects compared to nonsensitised animals). Lung function: Increased bronchial hyperreactivity. Sensitised animals also had increased lymphocytes in BAL, goblet cell hyperplasia, fibrosis and hydroxylproline in lung tissue, and transient increases of eosinophils in BAL, and IFNγ and TGFβ in lung tissue.	Hogaboam et al. (2000)

Table 9. Continued on next page

Table 9. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./group[a]	Exposure: Route	Exposure: Dose as specified in the study	Exposure: Dose estimated as spores/g bw[b]	Observation time	Effects	Reference
Aspergillus fumigatus									
	Mouse	Yes	4	i.t.	1×10^7 spores/animal	4×10^5	24 h		Hohl et al. (2005)
Heat-killed								BAL: No changes.	
Heat-killed, germinated								BAL: Increased neutrophils.	
Viable								BAL: Increased neutrophils.	
	Rabbit	No	25	i.t.	100–200 mg spores/animal	2×10^5–5×10^5	8 d–9 wk	Precipitins against *A. fumigatus.*	Morin et al. (1974)
5 strains	Rat	No	3	i.t.	1×10^9 spores/animal	6×10^6	25 wk	Mortality in different strains: 0/3–3/3. Symptoms: Dyspnoea and tachypnoea. Lung histology: Large regions with PMN inflammation, granulomas with basophils, hyphae, and necrosis; granulomas with PMN and spores; granulomas with AM, epithelial and Langhans cells; increased basophils and PMN.	Land et al. (1989)
Aspergillus terreus									
	Rabbit	No	3/6	ae	4×10^4-3×10^5 cfu/g lung	4×10^2–3×10^3	24 h	Blood: Dose-related decrease in PaO_2 and complement.	Olenchock et al. (1976)
	Rabbit	No	4	ae	3×10^5 cfu/g lung	4×10^3	4 h	Blood: Decreased PaO_2 and platelet counts.	Burrell & Pokorney (1977)
	Rabbit	No	12–16	ae	4×10^5 cfu/g lung	6×10^3	24 h	Blood: Decreased PaO_2 and increased haptoglobin.	Baseler & Burrell (1981)
	Rabbit	No	8–14	ae	80 mg spores/m^3, 30 min	2×10^5	24 h	Blood: Decreased PaO_2, no change in $PaCO_2$, temporarily increased white blood cells, no change in platelets, increased PMN/lymphocytes ratio. Lung histology: No change.	Olenchock et al. (1979)
	Rabbit	Yes	2	i.t.	7×10^6 spores/animal	4×10^3	2 h	Lung histology: No inflammation.	Olenchock et al. (1983)
	Rat	No	Not specified	i.t.	7×10^6 spores/animal	4×10^3	48 h	Lung histology: Granuloma-like lesions with AM, central necrosis and free spores.	Green et al. (1980)
	Rat	No	2	i.t.	5×10^7 spores/animal	1×10^5	1 wk	Lung histology: Formation of granuloma with spores, AM, PMN, and lymphocytes.	Olenchock et al. (1983)
Aspergillus versicolor									
	Mouse	Yes	3–10	i.t.	5×10^6 spores/animal	2×10^5	28 d	BAL: Transiently increased TNFα, IL-6, neutrophils. Lung histology: Transient inflammation with neutrophils, macrophages, and lymphocytes, and some granulomas.	Jussila et al. (2002b)
Aspergillus versicolor									
	Mouse	Yes	3–10	i.t.	10^5–10^8 spores/animal	1×10^4–4×10^6	24 h	BAL: Dose-related increase in TNFα, IL-6, neutrophils, AM, albumin, lactate dehydrogenase, and haemoglobin. Lung histology: Dose-related inflammation with neutrophils, macrophages, and lymphocytes.	Jussila et al. (2002b)

Table 9. Continued on next page

Table 9. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./group[a]	Exposure: Route	Exposure: Dose as specified in the study	Exposure: Dose estimated as spores/g bw[b]	Observation time	Effects	Reference
Cladosporium cladosporoides									
	Mouse	Yes	5	i.t.	3×10^{1}–3×10^{3} spores/g bw	3×10^{1}–3×10^{3}	96 h	BAL: Albumin, lactate dehydrogenase, IL-1β not significantly changed. Dose-related temporary increase in IL-6 and TNFα.	Flemming et al. (2004) (57)
	Mouse	Yes	Not specified	i.t.	5×10^{5} spores	2×10^{4}	72 h	BAL: Minor changes in surfactant production.	Mason et al. (1998)
	Mouse	Yes	5–6	i.t.	1×10^{6} spores	7×10^{4}	96 h	Lung histology: Granuloma formation with AM, PMN increasing with observation time. Transiently reduced alveolar air space.	Rand et al. (2003)
Cladosporium herbarum									
	Mouse	No	5	i.n.	2×10^{5} spores	1×10^{3}	24 h	*Nonsensitised animals.* BAL: No changes. *Sensitised animals.* BAL: Increased AM, neutrophils, eosinophils, lymphocytes, IL-4, IL-5, and IL-13.	Havaux et al. (2005)
Penicillium sp.									
	Rabbit	No	3	ae	3×10^{5} cfu/g lung	4×10^{3}	3 wk	Lung histology: No changes. Transient ungerminated spores in AM in lung tissue.	Thurston et al. (1979)
Penicillium spinulosum									
	Mouse	Yes	3–10	i.t.	5×10^{6} spores/animal	2×10^{5}	28 d	BAL: Transient increases in neutrophils and monocytes. Lung histology: Transient inflammation with neutrophils and monocytes.	Jussila et al. (2002a)
Penicillium spinulosum									
	Mouse	Yes	3–10	i.t.	1×10^{5}–5×10^{7} spores/animal	1×10^{4}–2×10^{6}	24 h	BAL: Increased TNFα, IL-6, and neutrophils, decreased macrophages. Lung histology: Dose-related neutrophilic inflammation.	Jussila et al. (2002a)
Penicillium aurantiogriseum									
	Guinea pig	No	Not specified	ae	1×10^{9} spores/m^3, 4 h	1×10^{5}	4 h	BAL: No changes in AM, lymphocytes, neutrophils and eosinophils. Lung histology: No change.	Fogelmark et al. (1991)
Phanerochaete chrysosporium									
	Guinea pig	No	Not specified	ae	2×10^{9} spores/m^3, 4 h	2×10^{5}	4 h	BAL: No changes in AM, lymphocytes, neutrophils and eosinophils. Lung histology: No change.	Fogelmark et al. (1991)
Rhizopus stolonifera									
	Guinea pig	No	Not specified	ae	2×10^{3}	2×10^{7}spores/m^3,	4 h	BAL: No changes in AM, lymphocytes, neutrophils and eosinophils. Lung histology: No change.	Fogelmark et al. (1991)
Stachybotrys chartarum									
Low-toxic	Mouse	No	4	i.n.	1×10^{6} spores/animal	5×10^{4}	3 d	Weight increase. Lung histology: Moderate inflammation with neutrophils, macrophages, haemorrhage, and granulomas.	Nikulin et al. (1996)
Nontoxic	Rat	No	4	i.t.	3×10^{3}–3×10^{4} spores/g bw	3×10^{3}–3×10^{4}	24 h	BAL: Increased MPO, no other changes (see below). No weight change.	Rao et al. (2000b)

Table 9. Continued on next page

Table 9. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./group[a]	Exposure: Route	Exposure: Dose as specified in the study	Exposure: Dose estimated as spores/g bw[b]	Observation time	Effects	Reference
Stachybotrys chartarum									
Nontoxic	Rat pup	No	12	i.t.	1×10^5–8×10^5 spores/g bw	1×10^5–8×10^5	3–14 d	LD_{18}: 8×10^5 spores/g bw; dose-related reduction in growth rate. Lung histology: Increased AM, spores within AM, mild interstitial pneumonia. BAL: No changes in AM, lymphocytes, neutrophils, TNFα, IL-1β, and haemoglobin.	Yike et al. (2002)
Toxic	Mouse	No	4	i.n.	1×10^6 spores/animal	5×10^4	3 d	Weight loss. Lung histology: Severe inflammation with neutrophils, macrophages, haemorrhage, granuloma, and necrosis.	Nikulin et al. (1996)
Toxicity not specified	Mouse	Yes	2–4	i.t.	5×10^5 spores	2×10^4	72 h	BAL: Increased production of surfactant and accumulation of "used" surfactant.	Mason et al. (1998)
Toxic	Rat	No	4	i.t.	3×10^3–3×10^4 spores/ g bw	3×10^3–3×10^4	24 h	BAL: Dose-related increases in MPO, lactate dehydrogenase, haemoglobin, albumin, and PMN; no changes in AM, lymphocytes, and eosinophils. Dose-related weight loss.	Rao et al. (2000b)
Toxic	Rat	No	4	i.t.	10^7 spores/animal	3×10^4	3 d	BAL: Increases in LDH, haemoglobin, AM, lymphocytes, and eosinophils; transient increase in albumin and PMN; no changes in MPO. Weight loss.	Rao et al. (2000a)
Toxic	Rat pup	No	12	i.t.	1×10^5–8×10^5 spores/ g bw	1×10^5–8×10^5	3-14 d	LD_{50}: 2.7×10^5 spores/g bw; dose-related reduction in growth rate. Lung histology: Dose-related haemorrhage, increased AM, spores within AM, mild interstitial pneumonia. BAL: Transient increases in AM, lymphocytes, neutrophils, TNFα, IL-1β, and haemoglobin. Transient increase in airway resistance.	Yike et al. (2002)
Toxic	Mouse	Yes	5–6	i.t.	1×10^6 spores	7×10^4	96 h	Lung histology: Granuloma formation with AM, PMN increasing with observation time. Transient erythrocytes and hemosiderin formation. Decreased collagen labelling in granulomas compared to controls and *Cladosporium cladosporoides*. Reduced alveolar airspace.	Rand et al. (2003)
Toxic, trichothecene producing	Mouse	Yes	5	i.t.	3×10^1–3×10^3 spores/g bw	3×10^1-3×10^3	96 h	BAL: Dose-related increase in albumin, IL-1β, IL-6 (intermittent for IL-1β and for IL-6 at the highest dose). Increased TNFα (intermittent at lowest dose) and increased lactate dehydrogenase.	Flemming et al. (2004)
Toxic, atranone producing	Mouse	Yes	5	i.t.	3×10^1–3×10^3 spores/g bw	3×10^1-3×10^3	96 h	BAL: Dose-related increase in albumin, IL-1β, IL-6 (intermittent for IL-1β and for IL-6 at the highest dose). Increased TNFα (intermittent at lowest dose) and increased lactate dehydrogenase.	Flemming et al. (2004)

Table 9. Continued on next page

Table 9. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./ group[a]	Exposure: Route	Exposure: Dose as specified in the study	Exposure: Dose estimated as spores/g bw[b]	Observation time	Effects	Reference
Actinomycetes									
Saccharopolyspora rectivirgula									
	Guinea pig	No	Not specified	ae	3×10^8 spores/ m^3, 4 h	3×10^4	4 h	BAL: No changes in AM, lymphocytes. Increased neutrophils and eosinophils. Lung histology: No change.	Fogelmark et al. (1991)
	Guinea pig	No	30	i.t.	800 µg spores/ animal	4×10^6	26 wk	Lung histology: Transient changes: inflammation with AM and PMN; thickened alveolar septa.	Zaidi et al. (1971)
	Guinea pig	No	30	i.t.	700 µg spores/ animal	3×10^6	13 wk	Lung histology: Transient changes: increased PMN and AM; interstitial pneumonitis; slight fibrotic changes; lymph node infiltration by lymphocytes.	Zaidi et al. (1983)
Streptomyces californicus									
	Mouse	No	5–10	i.t.	2×10^7–3×10^8 spores/ animal	1×10^6–2×10^7	7 d	BAL: Transient increases in IL-6 and TNFα (both also in serum), AM, neutrophils, albumin, lactate dehydrogenase, haemoglobin, and total protein. Lymphocytes increased after 7 d, iNOS increased in BAL cells after 24 h only. Lung histology: Transient dose-dependent neutrophilic inflammation.	Jussila et al. (2001)
Streptomyces thermohygroscopicus									
	Rabbit and rat	No	ca. 10	i.t.	20 µg spores/g bw	4×10^7	7 d	Lung histology: Transient inflammation with PMN, AM, phagocytosis of spores by AM.	Che et al. (1989)

[a]Number of animals in exposed and control groups.
[b]Dose estimated as described in Section 10.1.
ae: aerosol; AM: alveolar macrophages; BAL: bronchoalveolar lavage; cfu: colony-forming units; IFNγ: interferon gamma; IL: interleukin; i.n.: intranasal; iNOS: inducible nitric oxide synthase; i.t.: intratracheal; $LD_{50\ (or\ 18)}$: lethal dose for 50% (or 18%) of the animals at single exposure; MCP: monocyte chemoattractant protein; MPO: myeloperoxidase; $PaCO_2$: arterial carbon dioxide tension; PaO_2: arterial oxygen tension; PMN: polymorphonuclear leukocytes; TGFβ: transforming growth factor beta; TNFα: tumour necrosis factor alpha.

2002b; Flemming et al., 2004). Myeloperoxidase increased after exposure to toxic and nontoxic *S. chartarum* (Rao et al., 2000b). The responses to *A. versicolor, Cladosporium cladosporoides,* nontoxic and toxic *S. chartarum* were dose related (Rao et al., 2000b; Jussila et al., 2002b; Flemming et al., 2004). In studies of *A. alternata* and *C. herbarum,* increases in IL-4, IL-5, and IL-13 in sensitised mice were also reported, indicative of an allergic response (Havaux et al., 2005). The increased levels generally returned to normal within 3–28 days (Rao et al., 2000a; Jussila et al., 2001, 2992b;Yike et al., 2002; Flemming et al., 2004).

One study of specific pathogen–free mice that were intratracheally exposed to *A. fumigatus* indicated that viable spores induced a large increase of neutrophils in bronchoalveolar lavage fluid, which was not observed when heat-killed spores were applied. However, when spores were incubated until swollen before heat-killing, a similar inflammatory response was seen as induced by viable spores (Hohl et al., 2005). The applied dose (1×10^7 spores/animal, approximately 4×10^5 spores/g bw) was high compared to the doses used in the other studies. The study indicates that agents produced during germination can be responsible for the inflammatory response.

Increased surfactant production was observed after exposure of specific pathogen–free mice to toxic *S. chartarum,* but only minor changes after exposure to *C. cladosporoides* (Mason et al., 1998). These results indicate that alveolar type II cells are involved, but it is not clear how these changes contribute to the inflammatory process.

Lung tissue. In many studies of lung tissue, increases in inflammatory cells, mainly alveolar macrophages, lymphocytes, and polymorphonuclear leukocytes (PMNs), were observed. These studies included *Aspergillus fumigatus, Aspergillus versicolor, Penicillium spinulosum, Saccharopolyspora rectivirgula, Streptomyces californicus, Streptomyces thermohygroscopicus,* and toxic and nontoxic *Stachybotrys chartarum,* and exposed Guinea pigs (Zaidi et al., 1971, 1983), rabbits (Che et al., 1989), rats (Che et al., 1989; Land et al., 1989; Hogaboam et al., 2000; Yike et al., 2002), and mice (Nikulin et al., 1996; Jussila et al., 2001,

2002a, 2002b), of which two studies used specific pathogen–free animals (Jussila et al., 2002a, 2002b). The increase in inflammatory cells was observed after ≥3 days and was temporary in most studies within observation times of 1–26 weeks. Exposure levels were often high, 10^5 to 4×10^7 spores/g bw, and overload of the alveolar macrophages is likely. One study of *A. fumigatus* and two studies of toxic and nontoxic *S. chartarum* showed inflammation that did not return to normal. However, the applied doses were so high that mortality was observed in two of the studies (Land et al., 1989; Nikulin et al., 1996; Yike et al., 2002). In three studies, histopathological changes in lung tissue were dose related (Jussila et al., 2001, 2002a; Yike et al., 2002).

No inflammation of lung tissue by formation of granuloma and fibrosis was observed within 4 hours after exposure to *A. fumigatus, Aspergillus terreus, Penicillium aurantiogriseum, Phanerochaete chrysosporium, Rhizopus stolonifera,* and *S. rectivirgula* in pathogen-free rabbits (Olenchock et al., 1983) and Guinea pigs (Fogelmark et al., 1991). The doses were 10^2 to 2×10^5 spores/g bw and were applied by inhalation, except for *A. terreus*, which was instilled intratracheally. However, granuloma formation was observed after intratracheal instillation of *A. terreus, A. fumigatus, A. versicolor, Cladosporium cladosporoides,* and toxic and nontoxic *S. chartarum* spores in rabbits, rats, mice, and specific pathogen–free mice (Green et al., 1980; Kurup & Sheth, 1981; Olenchock et al., 1983; Land et al., 1989; Nikulin et al., 1996; Jussila et al., 2002b; Rand et al., 2003), and aerosol exposure of rabbits to *A. fumigatus* (Thurston et al., 1979). Most granulomas contained spores, alveolar macrophages, and PMNs, but also granulomas with eosinophilic and basophilic granulocytes, germinating spores, and hyphae were observed in two studies of *A. fumigatus* (Thurston et al., 1979; Land et al., 1989). Granuloma formation was temporary in three studies (Thurston et al., 1979; Kurup & Sheth, 1981; Jussila et al., 2002b) with observation times of 3–4 weeks, whereas the other studies were shorter (1 week or less) except one high-level exposure study in which mortality was observed (Land et al., 1989). It seems therefore possible that granuloma formation may regress within a few weeks unless exposure is extremely high. The inhaled dose of *A. fumigatus* in the study by Thurston et al. (1979), 2×10^4 spores/g bw, was estimated by culture and may have been more similar to the intratracheally applied dose by Land et al. (1989), 6×10^6 spores/g bw. In all studies, except the study by Green et al. (1980), doses of $\geq1\times10^5$ spores/g bw were applied. Macrophage overload can therefore not be ruled out at least locally due to intratracheal instillation.

In sensitised mice, eosinophil counts in lung tissue increased transiently after intratracheal exposure to 2×10^5 *A. fumigatus* spores/g bw and fibrosis developed. The eosinophilic inflammation in lung tissue was much weaker and no fibrosis was observed in nonsensitised mice (Hogaboam et al., 2000). However, Thurston et al. (1979) observed eosinophils in nonsensitised rabbits after inhalation exposure to *A fumigatus* spores. The applied dose was probably much higher than 2×10^4 spores/g bw that was reported (see previous paragraph).

A special finding in studies of nontoxic and toxic *S. chartarum* is the presence of erythrocytes and haemorrhage in the lung as well as increased haemoglobin levels (Nikulin et al., 1996; Rao et al., 2000a, 2000b; Yike et al., 2002; Rand et al., 2003).

Two studies of specific pathogen–free mice exposed to *A. fumigatus* showed increased levels of the inflammatory markers IL-3, IL-4, IL-18, IFNγ, transforming growth factor beta (TGFβ), and monocyte chemoattractant protein (MCP)-1/CCL2 in lung tissue and a decreased level of IL-10 (Hogaboam *et al., 2000; Blease et al.,* 2001). Most changes returned to baseline levels within an observation time of 4 weeks.

Zaidi et al. (1971, 1983) exposed Guinea pigs to *Saccharopolyspora rectivirgula* spores (3×10^6 to 4×10^6 spores/g bw) and 75 mg hay dust or bagasse. More intense cellular inflammation was observed in lung tissue after exposure to hay dust combined with *S. rectivirgula* than after exposure to the agents alone. The inflammation had cleared after 30 days. Fibrosis developed after exposure to hay dust with and without *S. rectivirgula* with no differences between the two exposure regimes, but no fibrosis occurred after exposure to *S. rectivirgula* alone (Zaidi et al., 1971). Bagasse dust alone induced small granulomas, *S. rectivirgula* alone induced pneumonitis, and interstitial fibrosis developed after combined exposure to these agents (Zaidi et al., 1983).

These results suggest that inflammation in lung tissue develops after more than 4 hours. However, the studies with short observation time applied lower doses than those of longer duration. Intratracheal instillation was mainly applied, and overload may have contributed to the inflammatory effects. Most changes returned to normal within a few weeks except after very high doses. Three species of actinomycetes and eight fungal species showed inflammatory responses, including *A. fumigatus* and toxic and nontoxic *S. chartarum*. Signs of eosinophilic inflammation were observed after exposure to *A. fumigatus* in three studies.

Bagasse dust but not hay dust seems to interact with *S. rectivirgula*. The applied doses of hay and bagasse dust correspond to approximately 300 μm^3/alveolar macrophage assuming that Guinea pigs have the same number of alveolar macrophages as rats (2.5×10^8). This dose exceeds by far the overload limit of 60 μm^3/alveolar macrophage suggested by Morrow (1992) (Sections 8.1 and 10.1). Overload conditions are therefore very likely in these studies and such exposure levels seem unlikely in the work environment. The observed interaction effects may thus be irrelevant.

10.4.3. Blood gas parameters

Arterial oxygen pressure and other blood parameters were measured in four studies using rabbits exposed to airborne *Aspergillus terreus* spores at levels from 4×10^2 to 2×10^5/g bw. The highest level was estimated from the applied aerosol (Olenchock et al., 1979). In the other studies, exposure levels were underestimated because lung tissue was cultured

after aerosol exposure (Olenchock et al., 1976; Burrell & Pokorney, 1977; Baseler & Burrell, 1981). Decreased arterial oxygen pressure as well as reductions in platelet counts, haptoglobin, and complement were observed, except in one study where platelet counts were unchanged (Olenchock et al., 1976, 1979; Burrell & Pokorney 1977; Baseler & Burrell, 1981). A dose-response association for arterial oxygen pressure was found in one study. The latter study also included *Aspergillus fumigatus,* which reduced arterial oxygen pressure after exposure to a 10-fold lower dose than *A. terreus* (Olenchock et al., 1976). The dose estimated from the applied aerosol was probably below the level where macrophage overload would occur (approximately 10^6 spores/g bw for the 2–2.5-µm spores of *A. terreus*).

10.4.4. Lung function

Only two studies included lung function parameters. Bronchial hyperreactivity developed both in mice sensitised to *Aspergillus fumigatus* and in nonsensitised mice after intratracheal exposure to *A. fumigatus* spores (Hogaboam et al., 2000). Airway resistance increased in rat pups intratracheally exposed to spores of toxic *Stachybotrys chartarum* but not after exposure to nontoxic spores (Yike et al., 2002). These changes returned to normal within 2–4 weeks except in sensitised mice exposed to *A. fumigatus*.

In one study, respiratory symptoms were reported. Land et al. (1989) exposed rats intratracheally to a single dose of *A. fumigatus* of five different strains. The animals developed dyspnoea and tachypnea after exposure to four of the strains and clear differences were observed between the fungal strains. Gliotoxin was not detected in the spores any of these strains.

In all studies, intratracheally administered spores from pathogenic species were used and relatively high doses were applied ranging from 1×10^5 to 6×10^6 spores/g bw.

10.4.5. Discussion

Inflammatory changes in bronchoalveolar lavage and lung tissue, lung function changes, and mortality were observed after exposure to diverse fungal and actinomycete species in different types of laboratory animals. In several studies, overload of the alveolar macrophages is a problem, which may have aggravated the response. However, such high exposure levels have been measured in relation to attacks of ODTS (Sections 8.1 and 11.4.2).

In general, most effects were temporary and returned to baseline levels within weeks in spite of possible overload. *A. fumigatus* and toxic *S. chartarum* generally elicited stronger responses than other species or nontoxic *S. chartarum* spores in comparative studies. Dose-effect relationships were demonstrated in many studies of various species, mainly for changes in inflammatory cells and markers in bronchoalveolar lavage and inflammatory changes in lung tissue. The dose-effect relationships in some of the studies may have been generated artificially due to local aggregation of spores after intratracheal instillation (Section 10.1).

Granulomas in lung tissue contained spores, alveolar macrophages, neutrophils, and lymphocytes. However, after exposure to *A. fumigatus,* signs of allergic inflammation were also observed, as indicated by granulomas with eosinophils, increases in eosinophils and IL-4 in lung tissue, and increases in serum IgE. Increases in eosinophils were also found in bronchoalveolar lavage after exposure to toxic *S. chartarum* spores, and to *A. alternata* and *C. cladosporoides* in sensitised animals.

One study addressed effects on lung surfactant in bronchoalveolar lavage. Toxic *S. chartarum* induced stronger increases than *C. cladosporoides.* These results indicate that alveolar type II cells are involved in the inflammatory process but it is not clear how.

10.5. Effects of short-term exposures (up to 90 days)

Seven short-term exposure studies have been found that are summarised in Table 10. The one study that applied aerosol exposure is also described in the text.

Fogelmark *et al.* (1991) exposed Guinea pigs (n=not given) 4 hours per day on 5 days per week during 3 and 5 weeks to dry aerosols of *Aspergillus fumigatus* (7×10^5 and 3×10^7 spores/m^3), *Rhizopus stolonifera* (2×10^7 spores/m^3), *Phanerochaete chrysosporium* (2×10^9 spores/m^3), *Penicillium aurantiogriseum* (1×10^9 spores/m^3), and *Saccharopolyspora rectivirgula* (3×10^8 spores/m^3). The animals were sacrificed 24 hours after the last exposure. Spore concentrations were quantified by microscopic counting of filter samples. Alveolar macrophage, neutrophil, eosinophil, and lymphocyte counts increased in bronchoalveolar lavage fluid for all examined species, except alveolar macrophage and neutrophil counts after exposure to the lowest dose of *A. fumigatus*. The strongest responses were observed for *P. aurantiogriseum* followed by *S. rectivirgula*. Lung histology revealed changes ranging from slight cell infiltration of the alveoli after exposure to the lowest dose of *A. fumigatus* to severe cell infiltration in alveoli and interstitial tissue, alveolar wall thickening, and granuloma formation after exposure to *P. aurantiogriseum* and the highest dose of *A. fumigatus.*

The Fogelmark et al. (1991) study is the most important study, as it is the only study in which animals were exposed to spore aerosols. The exposure pattern was not very different from workplace exposure, and five species were included, including *A. fumigatus* at two levels. Exposure levels may have been underestimated because spores were washed off cellulose ester filters before microscopic counting, and such filters are not suitable for resuspension of collected particles. The weakest responses were observed after exposure to 7×10^5 spores/m^3 of *A. fumigatus,* namely, slight signs of lung inflammation. Responses to other species were stronger, but exposure levels were higher and varied between species. However, some generalisations can be made: (1) spores from all species induced increased counts of alveolar macrophages, lymphocytes, neutrophils, and eosinophils in bronchoalveolar lavage fluid; (2) all spore types induced histopathological changes; (3) the responses did not decline within the observation period; (4) the high exposure level

Table 10. Animal experiments with repeated exposures to spores from fungi and actinomycetes.

	Animals			Exposure					
Microorganism	Species	Specific pathogen free	No./ group	Route	Dose as specified in the study, spores	Dose estimated as spores/g bw	Frequency	Effects	Reference
Fungi									
Alternaria alternata									
	Mouse	No	5	i.n.	2×10^5	1×10^3	1/d for 3d	*Nonsensitised animals.* BAL: Increased eosinophils. Lung histology: Neutrophilic inflammation. Lung tissue: Up-regulated eotaxin, MIP-1α, MIP-2, and MCP-1 receptors. *Sensitised animals.* BAL: Increased AM, lymphocytes, neutrophils, and eosinophils. Lung histology: Neutrophilic and eosinophilic inflammation. Alveolar wall thickening and goblet cells. Lung tissue: Upregulated eotaxin, MIP-1α, MIP-2, and MCP-1 receptors. Airway hyperreactivity.	Havaux et al. (2005)
Aspergillus fumigatus									
	Guinea pig	No	Not specified	ae	$7\times10^5/m^3$, 4 h	60	5 d/wk for 5 wk	BAL: No change in AM and neutrophils, increased lymphocytes and eosinophils. Lung histology: Slight cell infiltration of the alveoli. All after 3 and 5 w.	Fogelmark et al. (1991)
	Guinea pig	No	Not specified	ae	$3\times10^7/m^3$, 4 h	3×10^3	5 d/wk for 5 wk	BAL: Increased AM, neutrophils, lymphocytes and eosinophils. Lung histology: Cell aggregation in alveoli, alveolar wall thickening with interstitial cells and granuloma formation. All after 3 and 5 wk.	Fogelmark et al. (1991)
Cladosporium herbarum									
	Mouse	No	5	i.n.	2×10^5	1×10^3	1/d for 3d	*Nonsensitised animals*. BAL: No changes. Lung histology: No changes. Lung tissue: Upregulated eotaxin, MIP-1α, MIP-2, and MCP-1 receptors. *Sensitised animals.* BAL: Increased AM, neutrophils, eosinophils and lymphocytes. Lung histology: Neutrophilic and eosinophilic inflammation. Alveolar wall thickening and goblet cells. Lung tissue: Upregulated eotaxin, MIP-1α, MIP-2, and MCP-1 receptors. Airway hyperreactivity.	Havaux et al. (2005)
Penicillium chrysogenum									
Nonviable	Mouse	No	6	i.n.	1×10^4	7×10^2	1/wk for 6 wk	Blood: Increased total IgG_{2a}, neutrophils; decreased specific IgE and IgG_{2a}. BAL: Increased IFNγ. Lung histology: Lymphoid aggregates.	Cooley et al. (2000)

Table 10. Continued on next page

Table 10. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./ group	Exposure: Route	Exposure: Dose as specified in the study, spores	Exposure: Dose estimated as spores/g bw	Frequency	Effects	Reference
Penicillium chrysogenum									
Viable	Mouse	No	6	i.n.	1×10^4	7×10^2	1/wk for 6 wk	Blood: Increased total IgE, specific IgE and IgG_1, eosinophils; decreased specific IgG_{2a}. BAL: Increased eosinophils, IL-4, IL-5. Lung histology: Lymphoid aggregates + some eosinophils.	Cooley et al. (2000)
Nonviable	Mouse	No	5–8	i.n.	1×10^2	7	1/wk for 11 wk	*Nonsensitised animals.* Blood: Unchanged total IgE, IgG_1 and IgG_{2a}. BAL: No production of cytokines. Lung histology: No inflammation. *Sensitised animals.* Blood: Unchanged total IgE, IgG_1 and IgG_{2a}. BAL: No production of cytokines. Lung histology: No inflammation.	Schwab et al. (2003)
Viable	Mouse	No	5–8	i.n.	1×10^2	7	1/wk for 11 wk	*Nonsensitised animals.* Blood: Unchanged total IgE, IgG_1 and IgG_{2a}. BAL: No production of cytokines. Lung histology: No inflammation. *Sensitised animals.* Blood: Increased total IgE and IgG_1. BAL: Increased eosinophils, no production of cytokines. Lung histology: Eosinophilic and neutrophilic inflammation.	Schwab et al. (2003)
Penicillium aurantiogriseum									
	Guinea pig	No	Not specified	ae	$1\times10^9/m^3$, 4 h	1×10^5	5 d/wk for 5 wk	BAL: Increased AM, neutrophils, lymphocytes and eosinophils. Lung histology: Defined granulomas. All after 5 wk.	Fogelmark et al. (1991)
Phanerochaete chrysosporium									
	Guinea pig	No	Not specified	ae	$2\times10^9/m^3$, 4 h	2×10^5	5 d/wk for 5 wk	BAL: Increased AM, neutrophils, lymphocytes and eosinophils. Lung histology: Severe cell infiltration in alveoli and interstitial tissue, alveolar wall thickening and granuloma formation. All after 5 wk.	Fogelmark et al. (1991)
Rhizopus stolonifera									
	Guinea pig	No	Not specified	ae	$2\times10^7/m^3$, 4 h	2×10^3	5 d/wk for 5 wk	BAL: Increased AM, neutrophils, lymphocytes and eosinophils. Lung histology: Cell infiltration in alveoli and interstitial tissue, alveolar wall thickening. All after 3 and 5 wk.	Fogelmark et al. (1991)

Table 10. Continued on next page

Table 10. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./group	Exposure: Route	Exposure: Dose as specified in the study, spores	Exposure: Dose estimated as spores/g bw	Frequency	Effects	Reference
Stachybotrys chartarum									
Nontoxic	Mouse	No	7	i.n.	1×10^3	50	2/wk for 3 wk	Blood: Increased eosinophils, red blood cells, haemoglobin, haematocrit, and lymphocytes. Lung histology: No changes. No HPC in other organs.	Nikulin et al. (1997)
Nontoxic	Mouse	No	7	i.n.	1×10^5	5×10^3	2/wk for 3 wk	Blood: Increased eosinophils, red blood cells, haemoglobin, and haematocrit. Lung histology: Mild to moderate inflammation. No HPC in other organs.	Nikulin et al. (1997)
Toxic	Mouse	No	3	i.n.	1×10^3	50	2/wk for 3 wk	Blood: Increased platelets and haematocrit. Lung histology: Mild to moderate inflammation. No HPC in other organs.	Nikulin et al. (1997)
Toxic	Mouse	No	8	i.n.	1×10^5	5×10^3	2/wk for 3 wk	Blood: Increased eosinophils, red blood cells, haemoglobin, and haematocrit. Lung histology: Severe inflammation with AM, neutrophils and lymphocytes associated with spores. No HPC in other organs.	Nikulin et al. (1997)
Actinomycetes									
Saccharopolyspora rectivirgula									
	Guinea pig	No	Not specified	ae	$3\times10^8/m^3$, 4 h	3×10^4	5 d/wk for 5 wk	BAL: Increased AM, neutrophils, lymphocytes and eosinophils. Lung histology: Cell infiltration in alveoli and interstitial tissue, alveolar wall thickening. All after 3 and 5 wk.	Fogelmark et al. (1991)
Streptomyces californicus									
	Mouse	No	16	i.t.	2×10^3–2×10^7/animal	1×10^6–2×10^7	1/wk for 6 wk	BAL: Dose-related increase in AM, neutrophils, albumin and lactate dehydrogenase. No change in TNFα, IL-6. Lung tissue: Dose-related increase in activated T cells and non-T lymphocytes.	Jussila et al. (2003)
Streptomyces thermohygroscopicus									
	Rabbit	No	ca. 5	i.t.	20 µg/g bw	4×10^7	1/wk for 2–3 wk	Lung histology: Increased AM, granuloma with AM, epithelial giant cells and lymphocytes.	Che et al. (1989)
	Rat	No	ca. 5	i.t.	20 µg/g bw	4×10^7	1/wk for 2–3 wk	Lung histology: AM, granuloma with AM, epithelial giant cells and lymphocytes.	

Table 10. Continued on next page

Table 10. Continued.

Microorganism	Animals: Species	Animals: Specific pathogen free	Animals: No./ group	Exposure: Route	Exposure: Dose as specified in the study, spores	Exposure: Dose estimated as spores/g bw	Frequency	Effects	Reference
Streptomyces thermohygroscopicus									
	Rabbit	No	ca. 10	i.t.	20 µg/g bw	4×10^7	1/wk for 7–10 wk	Lung histology: AM, lymphocytes, fibrosis, less granulomas than after exposure for 2-3 wk that regressed over time.	Che et al. (1989)
	Rat	No	ca. 10	i.t.	20 µg/g bw	4×10^7	1/wk for 7–10 wk	Lung histology: AM, lymphocytes, fibrosis, less granulomas than after exposure for 2–3 wk that regressed over time.	

ae: aerosol; AM: alveolar macrophages; BAL: bronchoalveolar lavage; HPC: histopathological changes; Ig: immunoglobulin; IFNγ: interferon gamma; IL: interleukin;
i.n.: intranasal; i.t.: intratracheal; MIP: macrophage inflammatory protein; MCP: monocyte chemoattractant protein; TNFα: tumour necrosis factor alpha.

of *A. fumigatus* induced stronger responses than the lower level; and (5) differences between species were observed: *A. fumigatus* was more toxic than *Rhizopus stolonifera* and *Phanerochaete chrysosporium,* and *Penicillium aurantiogriseum* was more toxic than *P. chrysosporium.* The lowest observed effect level (LOEL) was 7×10^5 *A. fumigatus* spores/m^3, which corresponds to 4×10^5 spores/m^3 for an 8-hour time-weighted average exposure level.

Dose-related lung inflammation was observed in mice exposed intranasally to spores of *Stachybotrys chartarum* (50 and 5×10^3 spores/g bw applied twice weekly for 3 weeks). Spores with high levels of mycotoxins induced stronger inflammation than spores with low levels (Nikulin et al., 1997). No histopathological changes were observed in the thymus, spleen, or intestines, which are the target organs for trichothecene mycotoxins.

In mice intratracheally exposed to spores of the actinomycete *Streptomyces californicus* (1×10^2 to 1×10^6 spores/g bw applied weekly for 6 weeks), dose-related responses were found in bronchoalveolar lavage fluid for alveolar macrophages, neutrophils, albumin, and haemoglobin, and in lung tissue for activated T cells and non-T lymphocytes (Jussila et al., 2003).

Che et al. (1989) studied the effect of exposure duration in rabbits and rats that were intratracheally exposed to *Streptomyces thermohygroscopicus* (approximately 4×10^7 spores/g bw weekly during 7–10 weeks). Lung histology of both species showed increased numbers of inflammatory cells as well as granulomas after 2–3 weeks. The granulomas had regressed after 7–10 weeks but were followed by fibrosis. The exposure level in this study was higher than in any other study in Table 10 (based on spore numbers), but the observation time was also longer. The applied dose amounts to a volumetric dose of approximately 16 μm^3/alveolar macrophage in rats. This is below the overload limit of 60 μm^3/alveolar macrophage suggested by Morrow (1992) (Section 8.1) but intratracheal administration may still have created overload conditions locally in the lung (Sections 10.1 and 12.1).

Cooley et al. (2000) studied the role of spore viability in mice intranasally exposed to *Penicillium chrysogenum* (7×10^2 spores/g bw weekly for 6 weeks). Spores that had been killed by methanol treatment induced nonallergic responses in blood, with increased neutrophil counts and total IgG_{2a}, although specific IgG_{2a} and specific IgE decreased. In bronchoalveolar lavage, fluid IFNγ increased. Viable spores (25% viability) induced allergic responses in blood with increased total IgE, specific IgE, specific IgG_1, and eosinophils, and decreased specific IgG_{2a}. In bronchoalveolar lavage fluid, eosinophils, IL-4, and IL-5 increased, and also lung histology showed some eosinophils.

Schwab et al. (2003) extended the study by Cooley et al. (2000) to a 100 times lower dose (7 spores/g bw weekly for 11 weeks), spores were killed by formaldehyde vapour, and both nonsensitised mice and animals sensitised to *P. chrysogenum* were used. Inflammatory responses were only observed in sensitised mice exposed to viable spores in serum (increased IgE and IgG_1), bronchoalveolar lavage (increased eosinophils), and lung tissue (eosinophilic and neutrophilic inflammation).

Havaux et al. (2005) tested sensitised and nonsensitised mice with spores of *Alternaria alternata* and *Cladosporium cladosporoides*. In nonsensitised animals, both allergic (eosinophils in bronchoalveolar lavage [only *A. alternata*] and up-regulated eotaxin receptors in lung tissue) and nonallergic responses (neutrophilic inflammation and up-regulated receptors for MIP-1α, MIP-2, and MCP-1 in lung tissue) were found. In sensitised animals, more and stronger responses were observed as well as airway hyperreactivity. The responses to *A. alternata* spores were stronger than to *C. cladosporoides* but qualitatively similar.

Thus, nonallergic and allergic responses to fungal spores of eight species were observed after repeated exposures in seven studies (Che et al., 1989; Fogelmark et al., 1991; Nikulin et al., 1997; Cooley et al., 2000; Jussila et al., 2003; Schwab et al., 2003; Havaux et al., 2005). In one of those, the applied dose was 10 to >1000 times lower than in the other studies, and inflammatory responses, including allergic

responses in bronchoalveolar lavage, lung tissue and blood were observed in sensitised animals that had been exposed to viable spores (Schwab et al., 2003). As expected, prior sensitisation increased the intensity of allergic inflammation, but also nonallergic inflammation was increased. Dose-related responses were observed in all three studies that applied more than one dose level (Fogelmark et al., 1991; Nikulin et al., 1997; Jussila et al., 2003). The importance of viability of the spores was demonstrated in two of the studies (Cooley et al., 2000; Schwab et al., 2003) and it seems likely that at least a fraction of the spores were viable in the other studies although this was not mentioned. Two studies of two *Streptomyces* species showed only nonallergic inflammation (Che et al., 1989; Jussila et al., 2003), but these results cannot be generalised to actinomycetes because *Saccharopolyspora faeni* induced eosinophils in bronchoalveolar lavage fluid (Fogelmark et al., 1991).

In summary, repeated-exposure studies demonstrated long-lasting inflammatory changes in bronchoalveolar lavage fluid, lung tissue, and blood. Differences between species that partly depended on the presence of mycotoxins, and dose-related responses were generally observed.

Allergic responses to spores of various fungal species were observed in all studies. Viable spores induced allergic responses in contrast to nonviable spores. In one study with very low exposure, allergic and nonallergic inflammation was only observed in sensitised mice exposed to viable spores.

A single study showed that trichothecene mycotoxins in *S. chartarum* spores did not affect the target organs for these toxins after oral exposure.

A LOEL of 7×10^5 *A. fumigatus* spores/m^3 was observed in Guinea pigs in the only study applying aerosol exposure (4 hours) (Fogelmark et al., 1991).

10.6. Mutagenicity and genotoxicity

No studies have been found.

10.7. Effects of long-term exposure and carcinogenicity

Several mycotoxins are recognised carcinogens, e.g., the aflatoxins and ochratoxin A. Inhalation of spores containing the toxin as well as particles originating from the substrates with fungal growth represent potential sources of exposure to working populations (CAST, 2003). Experimental studies of the carcinogenicity of the pure substances have been carried out, but no studies of spores from fungi applied via the respiratory route have been found.

10.8. Reproductive and developmental studies

No studies have been found.

10.9. Discussion

Single- and repeated-exposure studies showed proinflammatory and inflammatory responses in the lung. In bronchoalveolar lavage fluid, increases in alveolar macrophages, PMNs as neutrophils and eosinophils, proinflammatory cytokines such as TNFα and IL-6, and markers of tissue damage as lactate dehydrogenase, albumin, and haemoglobin were observed. In lung tissue, alveolar macrophages, PMNs, lymphocytes, proinflammatory cytokines such as IL-3, IL-4, IL-18, TNFα, TGFβ, and MCP-1/CCL2 increased, IL-10 decreased, and granulomas were formed. Only one study observed fibrosis (90 days after repeated exposures to high doses of an actinomycete). A major difference between single- and repeated-exposure studies is that most changes were temporary after exposure to a single dose, whereas repeated exposures generally induced responses that persisted throughout the observation period.

Several single- and three repeated-exposure studies demonstrated dose-effect associations. However, in most studies the maximum applied doses were so high that overload seems likely. Only in the studies by Olenchock et al. (1976), Fogelmark et al. (1991), and Nikulin et al. (1997) were dose levels below 10^4 spores/g bw applied. These studies showed dose-effect associations between arterial oxygen tension and a single exposure to *Aspergillus terreus*, between inflammatory cells in bronchoalveolar lavage and inflammatory changes in lung tissue and prolonged exposure to aerosols of *A. fumigatus* spores, and between severity of lung inflammation and repeated intranasal exposures to toxic and nontoxic *Stachybotrys chartarum,* respectively. All tested species induced inflammatory effects but differences between species were observed in several studies. Species differences partly depended on mycotoxin production by the microorganism, as has been shown in several studies of *S. chartarum.* However, species not known as toxin producers, e.g., a strain of *A. fumigatus* not producing gliotoxin, *S. rectivirgula,* and *Penicillium aurantiogriseum,* also elicited strong responses. It cannot be ruled out, however, that microorganisms may produce toxins that have not yet been identified.

Many studies reported nonallergic inflammatory responses involving TNFα, IL-6, alveolar macrophages, and neutrophils. However, as the majority of the studies applied single exposures, development of an adaptive immune response was not possible. Interestingly, in all repeated-exposure studies including various fungi, allergic inflammation were observed together with nonallergic inflammation. Viable spores and prior sensitisation were important determinants of the allergic response (Cooley et al., 2000).

11. Observations in man

Studies with quantitative information on spore exposure, mainly obtained by culture-based methods and microscopic methods, are reviewed. Spore levels measured by culture methods were multiplied by a factor of 10 to estimate airborne spore levels, although the ratio spore/cfu varied from 1.5 to 100 in comparative studies; for a review see Eduard and Heederik (1998). Studies using specific serum IgG antibodies as a measure of exposure are excluded, as immunoglobulin levels are difficult to extrapolate to airborne spore levels. Human challenge studies using fungal extracts are included

as these studies provide information on the specificity of the response.

Case reports (human challenge studies) are summarised in Tables 11 and 12 and Appendix 8 of Eduard (2007), epidemiological studies of highly exposed populations in Table 13 (symptoms), Table 14 (objective outcomes), and Appendix 6 of Eduard (2007), and epidemiological studies of populations with common indoor exposure in Table 15 and Appendix 7 of Eduard (2007). Tables 13–15 include only studies where associations were adjusted for confounders. If no specific levels were reported for an increased response, the median of the reported exposure levels was used to indicate the exposure level where the outcome was increased.

Diseases are described shortly in "Terms as used in this document" and in more detail in Section 8.

11.1. Irritation

This section includes eye, nose, and throat irritation (Tables 13, 15). Symptoms and effects of the lower airways are reviewed in Sections 11.4 and 11.5.

11.1.1. Highly exposed populations

Irritation symptoms in the eyes and nose were recorded by a questionnaire simultaneously with exposure measurements in a study of 89 Norwegian farmers (Eduard et al., 2001). Exposure to many agents was measured, including fungal and actinomycete spores, bacteria, endotoxins, glucans, *Aspergillus/Penicillium* antigens, and silica. The prevalence of eye irritation was significantly elevated after exposure to 2×10^4 to 5×10^5 fungal spores/m^3 (odds ratio (OR) 8.3, 95% confidence interval (CI) 1.0–70 compared to exposure below 2×10^4 fungal spores/m^3) and was not associated with any of the other agents. The prevalence of nasal symptoms was elevated after exposure to 2×10^4 to 5×10^5 fungal spores/m^3 (OR 4.1, 95% CI 0.88–19) and significantly after exposure to 5×10^5 to 2×10^7 fungal spores/m^3 (OR 6.0, 95% CI 1.3–28). Nasal symptoms were also associated with silica exposure and both agents seemed to induce these symptoms, as the correlation between the two exposures was not very strong.

Work-related cough, nasal irritation, and sore throat have been studied by questionnaires in cross-sectional studies of wood workers in Norway and Australia, and were associated with fungal exposure (Eduard et al., 1994; Alwis et al., 1999). In wood trimmers, exposure to 2×10^6 spores/m^3 was associated with a 50% relative increase in self-reported cough, nasal irritation, and sore throat (Eduard et al., 1994). However, this study may suffer from reporting bias as work-related symptoms had been recorded conditional on

Table 11. Case-reports: Hypersensitivity pneumonitis confirmed by provocation testing.

Occupation/ environment	Species	Patient data	Challenge, BPT	Response	Reference
7 farmer lung patients working with mouldy hay	*Saccharopolyspora rectivirgula*	In addition to hypersensitivity pneumonitis, asthma ($n=4$) and bronchitis ($n=1$)	Whole culture extract	6 responded with fever, aches, malaise, and 5 showed FVC and FEV_1 decline.	Pepys et al. (1965)
16 farmers working with mouldy hay	*Saccharopolyspora rectivirgula*	X-ray ($n=14$), obstructive ($n=8$) and restrictive ($n=4$) lung function changes	Fungal extract	3 responded with fever, lung function changes (not specified).	Edwards & Davies (1981)
19 cork workers	*Penicillium glabrum*	No info on patient selection	Culture plate extract	Fever, cough, dyspnoea, malaise, crackles, TLco decline.	Ávila et al. (1974)
4 mushroom workers	*Pleurotus ostreatus*	Fever, chills, dyspnoea, dominated, cough in 2/4	Spore suspension (dose ca. 10^7 spores)	After 6–8 h, fever, chills, muscle pain, dyspnoea, leukocytosis, decreased FVC.	Cox et al. (1988)
5 mushroom workers	*Lentinus edodes*	Fever, chills, cough, dyspnoea, pain in muscles	Spore suspension (dose ca. 3×10^7 spores)	After 6 h, fever, chills, muscle pain, dyspnoea, leukocytosis, and decreased FVC. TLco and PaO_2 decreased in 1/5.	Cox et al. (1989)
17 patients with summer type hypersensitivity pneumonitis	*Trichosporon cutaneum*	Fever, cough, dyspnoea, IgG[a] T-lymphocytosis (BAL), granulomatous/hypersensitivity pneumonitis	Culture filtrate	16 responded with fever, cough, dyspnoea, rales, increase in white blood cells, often serotype specific.	Ando et al. (1990)
6 patients with hypersensitivity pneumonitis to wood-rot fungi	*Serpula lacrymans* ($n=3$) *Geotrichum candidum* ($n=2$) *Aspergillus fumigatus* ($n=2$)	Breathlessness, X-ray changes, and positive lung biopsy	Culture filtrate	Late reaction with ≥20% fall in TLco and/or FEV_1.	Bryant & Rogers (1991)

[a]Specific for the fungus used in the challenge.

BAL: bronchoalveolar lavage; BPT: bronchial provocation test; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; Ig: Immunoglobulin; PaO_2: arterial oxygen tension; TLco: lung transfer factor for carbon monoxide (measure of gas diffusion).

Table 12. Case reports: Asthma confirmed by provocation testing.

Occupation/ environment	Species	Patient data	Challenge, BPT	Response	Reference
4 asthmatic patients, occupation unknown	*Alternaria alternata*	Mild asthmatic, skin prick test[a]	Dry spores and extracts (positive response after 9×10^4 spores)	Immediate and late response (spores only), 35% decreased specific airway conductance.	Licorish et al. (1985)
3 asthmatic patients, occupation unknown	*Penicillium* sp.	Mild asthmatic, skin prick test[a]	Dry spores and extracts (positive response after 6×10^4 spores)	2 responded with immediate and late (spores only) 35% decreased specific airway conductance.	Licorish et al. (1985)
7 farmer's lung patients working with mouldy hay	*Saccharopolyspora rectivirgula*	Additional asthma ($n=4$) and bronchitis ($n=1$)	Whole culture extract	6 responded with fever, aches, malaise, and 5 showed FVC and FEV_1 decline.	Pepys et al. (1965)
16 farmers working with mouldy hay	*Saccharopolyspora rectivirgula*	X-ray ($n=14$), obstructive ($n=8$) and restrictive ($n=4$) lung function changes	Fungal extract	Immediate asthmatic response in 1 subject.	Edwards & Davies (1981)
3 tomato growers	*Verticillium albo-atrum*	Cough, wheeze, breathlessness, variable peak flow, (1/3 skin prick test positive[a], 0/3 IgE positive[a])	Mycelia extract	Immediate ($n=3$) and late ($n=2$) FEV_1 decline.	Davies et al. (1988)
6 employees from hospital with water damage (total population 14)	*Sporobolomyces salmonicolor*	Bronchial hyperreactivity (3/5), respiratory symptoms (4/6), skin prick test[a] (0/6), atopy (1/6)	Fungal extract	Doubling of airway resistance within 10 min ($n=3$) and >15% reduction in peak expiratory flow after 6 h ($n=1$), reversible by asthma medication, cough, dyspnoea and headache in ≥1.	Seuri et al. (2000)

[a]Specific for the fungus used in the challenge.
BPT: bronchial provocation test; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; Ig: immunoglobulin.

handling of mouldy timber. In joinery, sawmill, and chip mill workers, an increased prevalence of throat irritation was observed at an exposure level of 3×10^4 cfu/m^3 or 3×10^5 spores/m^3 (Alwis et al., 1999). The dominant fungi in these studies were *Rhizopus microsporus (*42%) and *Penicillium/ Aspergillus* species (71%), respectively.

In both studies of wood workers, associations were adjusted for age, smoking, and gender but not for atopy. Other relevant exposures such as wood dust (Eduard et al., 1994; Alwis et al., 1999) and endotoxins, glucans, and Gram-negative bacteria (Alwis et al., 1999) were either adjusted for (Eduard et al., 1994) or studied in separate models (Alwis et al., 1999). In the latter study, endotoxins were also associated with sore throat, but more weakly than fungal spores. Confounding of the association with fungal exposure is not likely because endotoxin exposure was only moderately correlated with fungal exposure. The farmer study (Eduard et al., 2001) is the most reliable as all relevant short-term exposure was measured. This study indicates LOELs for nose irritation of approximately 3×10^6 spores/m^3 (geometric mean of the range 5×10^5 to 2×10^7 spores/m^3), although the odds ratio was not significantly elevated after exposure to approximately 1×10^5 spores/m^3 (geometric mean of the range 2×10^4 to 5×10^5 spores/m^3), whereas eye irritation significantly elevated at approximately 1×10^5 spores/m^3. All exposure levels were calculated to equal 8-hour exposures, although the exposure time was 1 hour or less. The Australian sawmill study (Alwis et al., 1999) indicates a LOEL for sore throat of 3×10^5 spores/m^3 based on average exposure levels. No specific fungus was more potent in the data analysis.

11.1.2. Populations exposed to common indoor air

Li et al. (1997) studied 264 day-care workers from 28 randomly selected day-care centres in Taiwan. Nasal congestion and discharge were associated with total fungal levels, and nasal congestion also with *Aspergillus* levels (geometric means 1200 and 32 cfu/m^3, respectively; *n* not given). *Cladosporium* and *Penicillium* were the dominating species. Associations were adjusted for age, gender, and education, but not for smoking. House dust mite allergens were measured in settled dust but were not associated with any outcome. The study suggests that *Aspergillus* may be more irritating than other species, as *Aspergillus* comprised only a small fraction of all culturable fungi. Alternatively, this association might be due to a correlation with the total fungal count, as such correlations were not assessed.

Purokivi et al. (2001) compared eye symptoms in 37 employees from a school building with moisture problems and fungal growth with 23 employees from a school without such problems in Finland at the end of the spring term. Symptom prevalences were not significantly different. Fungal levels in the problem and nonproblem buildings

Table 13. Epidemiological studies of highly exposed populations: Symptoms associated with fungal spore exposure*.

Study group	Sampling method	Exposure level, spores/m^3	Association			Comments	Reference
			Symptom	Exposure level,[a] spores/m^3	OR (95% CI)		
89 farmers	Personal	2×10^3–2×10^7 (8-h TWA)	Nasal irritation	2×10^3–2×10^4 2×10^4-5×10^5 5×10^5-2×10^7	REF 4.1 (0.88–19) 6.0 (1.3-28)[b]	Current work-related symptoms recorded concomitantly with exposure measurements. Also adjusted for exposure to bacteria, endotoxins, and other agents.	Eduard et al. (2001)
			Eye irritation	2×10^3-2×10^4 2×10^4-5×10^5 5×10^5-2×10^7	REF 8.3 (1.0-70)[b] 7.0 (0.83-59)		
			Cough	2×10^3-2×10^4 2×10^4-5×10^5 5×10^5-2×10^7	REF 1.3 (0.28-5.7) 3.9 (1.0-15)[b]		
1614 farmers	Personal, Job exposure matrix[c]	2×10^5-4×10^7 (1-yr TWA)	Atopic asthma	2×10^5-2×10^6 2×10^6-4×10^6 4×10^6-4×10^7	REF 0.55 (0.23-1.3) 0.28 (0.10-0.78)[b]	Also adjusted for exposure to bacteria and endotoxins.	Eduard et al. (2004)
			Nonatopic asthma	2×10^5-2×10^6 2×10^6-4×10^6 4×10^6-4×10^7	REF 1.6 (0.96-2.6) 1.7 (1.0-2.7)[b]		
107 wood trimmers	Personal, group-based	4×10^5-2×10^7	Cough, morning Cough, WR Nasal obstruction, WR Sore throat, WR	2×10^6 2×10^6 2×10^6 2×10^6	1.5[b] 1.5[b] 1.5[b] 1.5[b]	Exposure levels that predict an OR of 1.5 in significant logistic regression models. Also adjusted for hayfever, and wood dust. Separate models with fungal species were also tested. Possible recording bias of work-related symptoms.	Eduard et al. (1994)
		0-7×10^6 *Rhizopus microsporus*	Shortness of breath, WR	2×10^6	1.5[b]		
951 waste collectors and 423 park workers (REF)	Personal, Job exposure matrix[c,d]	Not given	Chronic bronchitis	0 <2×10^5 ≥2×10^5	REF 1.9 (1.0-3.6)[b] 2.7 (0.7-11)[b,c]	Two groups of waste handlers were compared to a reference group. The exposure of the reference group was not measured and assumed to be 0. Only the cutpoint that divided two exposure categories of the waste handlers was reported.	Hansen et al. (1997)
950 waste collectors and 387 park workers (REF)	Personal, Job exposure matrix[d]	1×10^5-2×10^7 Weekly inhaled spore dose	Diarrhoea	0 1×10^5-1×10^6 >1×10^6-1×10^7 >1×10^7-2×10^7	REF 3.0 (1.9-4.9)[b] 3.5 (2.2-5.3)[b] 5.6 (2.4-13)[b]	Similar association with endotoxins. Three groups of waste handlers were compared to a reference group. The exposure of the reference group was not measured and assumed to be 0.	Ivens et al. (1999)

Table 13. Continued on next page

Table 13. Continued.

Study group	Sampling method	Exposure level, spores/m^3	Association			Comments	Reference
			Symptom	Exposure level,[a] spores/m^3	OR (95% CI)		
82 joinery workers and 108 saw-/chip mill workers	Personal	3×10^3-7×10^4 (arithmetic group means)	Throat irritation, WR Phlegm (joinery) Breathlessness, WR (saw-/chip mill)	3×10^4 1×10^4 4×10^4 (arithmetic means)	OR 1.6 (1.2-2.1)[b] 2.0 (1.0-3.9)[b] 4.8 (1.4-17)[b]	Exposures to allergenic wood dust, glucans, Gram-negative bacteria and endotoxins were evaluated in separate models. Models where fungi showed the strongest or independent relationships are shown.	Alwis et al. (1999)

*Associations have been adjusted for smoking, age, and gender. Studies are cross-sectional unless otherwise stated.
[a]Exposure level for which the association was calculated.
[b]Significantly different from the lowest exposed group (REF).
[d]Job exposure matrix: exposure estimated from information on performed tasks and other determinants, which had been validated by personal exposure measurements in a subset of the workers.
cfu: colony-forming units; CI: confidence interval; OR: odds ratio; PPR: prevalence proportion ratio; REF: reference group (OR or PPR =1); TWA: time-weighted average; WR: work-related.

Table 14. Epidemiological studies* of highly exposed populations: Objective outcomes associated with fungal spore exposure.

Study group	Sampling method	Exposure level, spores/m^3		Association		Comments	Reference
		Range	Median[a]	Endpoint	Outcome		
28 waste handlers	Personal	0–2×10^6 (8-h TWA)	2×10^5	% neutrophils in nasal lavage	Positive correlation	The influence of confounders was evaluated.	Heldal et al. (2003a)
			5×10^4 ($n=12$)	Δnasal volume after 3 days of exposure	Negative correlation		
25 waste collectors	Personal	0–2×10^6 (8-h TWA)	2×10^5	Change in inflammatory cells and mediators in induced sputum after 3 days of exposure	Not associated to exposure levels	The influence of confounders was evaluated.	Heldal et al. (2003b)
11 sawmill workers during work vs. vacation	Not specified	2×10^5–1.5×10^6 *Rhizopus* and *Penicillium* sp.	6×10^5	Inflammatory markers in nasal lavage	Not associated to exposure levels	The influence of confounders was not evaluated.	Roponen et al. (2002)
29 sawmill workers (follow-up)	Personal	1×10^2–4×10^6 cfu/m^3	2×10^4 cfu/m^3	ΔFVC after 4 d of exposure	Positive correlation	Stratified analysis in smoking categories. Design controls for age and gender.	Hedenstierna et al. (1986)

*Studies are cross-sectional unless otherwise stated.
[a] Central measure of the exposure distribution that showed an association with the outcome.
Δ: intraindividual change; cfu: colony-forming units; FVC: force vital capacity; TWA: time-weighted average.

in the winter were 29 and 6 cfu/m^3 (geometric mean), respectively. No confounder adjustments were carried out, however.

Roponen et al. (2003) studied nasal, laryngeal, and eye symptoms in 41 randomly selected Finnish teachers. Exposure to fungi was measured by personal sampling during 24 hours at home and 8 hours at work and the teachers were dichotomised into groups with low and high exposure. Exposure levels in these groups were 12 cfu/m^3 (median, range 0–31 cfu/m^3) and 67 cfu/m^3 (median, range 31–270 cfu/m^3), respectively. The prevalences of all symptoms were similar in the two groups. Exposure to bacteria

Table 15. Epidemiological studies* of working populations exposed to common indoor levels: Symptoms associated with fungal spore exposure.

Study group	Sampling method	Exposure level	Association: Work-related symptoms (effect)	Association: Outcome, OR (95% CI)	Comments	Reference
264 day-care workers from 28 randomly selected day-care centers	Area	1200 cfu/m³ (GM) *(Penicillium* and *Cladosporium* dominated, levels indoors slightly higher than outdoors)	Nasal congestion Nasal discharge	2.0 (1.2–3.3)[a] 1.7 (1.0–2.8)[a]	Exposure measurements poorly documented. Exposure variables in models not specified. Adjusted for age, gender, and education.	Li et al. (1997)
		32 *Aspergillus* cfu/m³ (GM)	Nasal congestion Cough Phlegm Lethargy Fatigue	1.7 (1.0–2.8)[a] 1.7 (1.1–2.8)[a] 1.8 (1.1–2.8)[a] 1.8 (1.0–3.1)[a] 2.0 (1.2–3.4)[a]	Separate models with other fungal species, total culturable bacteria, and house dust allergens on settled dust did not show associations.	
107 office workers with and 107 without work-related respiratory symptoms	Area in offices	0–7 *Alternaria* cfu/m³	Respiratory symptoms	4.2 (1.1–16)[a]	Levels of other species and total fungi not given. Subjects matched on age, gender, and atopy. Adjusted for smoking, mite allergen in house dust, and difference between absolute indoor and outdoor air humidity.	Menzies et al. (1998)
41 randomly selected teachers	Personal, home+work combined	0–270 cfu/m³	Irritation and nonspecific symptoms; nasal lavage: NO, IL-4, IL-6, TNFα, and IL-1β	Similar in groups with low (0–31 cfu/m³) and high (31–270 cfu/m³) exposure, except IL-1β that was nonsignificantly increased in the high exposure group	Exposure to bacteria was also studied. Confounder assessment by separate analyses in sub-groups based on asthma and smoking.	Roponen et al. (2003)

*Studies are cross-sectional unless otherwise stated.
[a]Significant association between exposure and symptom.
cfu: colony-forming units; CI: confidence interval; GM: geometric mean; IL: interleukin; OR: odds ratio; NO: nitric oxide; TNFα: tumour necrosis factor alpha.

was also measured. The influence of smoking and asthma status was evaluated by stratified analysis.

Thus, only the study by Li et al. found associations between nasal irritation and fungi (Li et al., 1997). This may be due to much higher exposure levels in this study compared to the other studies. Li et al. used random selection and controlled for confounders, which strengthen the reliability of their results, although the exposure assessment is poorly described. The geometric mean provides a LOEL estimate of approximately 1×10^4 spores/m³. The study by Roponen et al. (2003) has a strong design as both home and work exposure were assessed. The exposure level of the highest exposed group in this study, median 67 cfu/m³ or approximately 700 spores/m³, thus represent a no observed effect level (NOEL) for irritation.

11.2. Sensitisation

Sensitisation is often understood as the presence of serum IgE antibodies to a specific allergen. However, also hypersensitivity pneumonitis patients are more sensitive to exposure to allergens, which indicates that the hypersensitivity is not mediated by IgE. It is more likely that cytotoxic T cells are involved (Rose et al., 1996; Johansson et al., 2001; Patel et al., 2001; Section 8.2). The hypersensitivity can be shown by bronchial challenge to aerosolised fungal extracts and whole spores and such studies are therefore also included here.

11.2.1. Human challenge studies

Fungi and actinomycetes have been identified as causes of hypersensitivity pneumonitis and asthma by bronchial challenge of patients with the suspected antigens. Provocations have mainly been carried out with extracts of the organisms to avoid late responses that may be provoked by spores. Positive tests are strong evidence of work-related sensitisation when performed with specific microorganisms from the work environment. Challenge studies therefore provide important information about the aetiology of diseases associated with fungi.

Provocation tests were carried out with close surveillance of patients (Pepys et al., 1975), but are no longer recommended because of the risk of severe late reactions and lack of standardised antigens (Richerson et al., 1989; Rose et al., 1996). Exposure challenge to the workplace atmosphere is regarded sufficient to demonstrate an association with the disease, but identification of the causal agent(s) is difficult when the exposure is complex.

The focus of this section is on species that may cause sensitisation; the applied doses are discussed in Section 11.4.1.

Table 11 summarises studies on hypersensitivity pneumonitis including more than a single case. The provocations were carried out with spores or extracts of the implicated organism, or by exposure to work environments with one dominating species. A relevant nonoccupational study (Ando *et al.*, 1990) is also included in the table.

Studies reporting a single case identified *Aspergillus clavatus, Aspergillus glaucus, Lentinus edodes, Aspergillus fumigatus, Saccharomonospora viridis, Scopulariopsis brevicaulis,* and *Ustilago esculenta* as cause of hypersensitivity pneumonitis in a malt worker (Riddle et al., 1968), two mushroom workers (Yoshida et al., 1990; Matsui et al., 1992), a greenhouse worker (Yoshida et al., 1993), a logging worker who also raised pigs (Greene et al., 1981), a tobacco worker (Lander et al., 1988) and a traditional handicraft worker (Yoshida et al., 1996), respectively.

The case studies show that hypersensitivity pneumonitis can be induced by several species of *Aspergillus* and *Penicillium* as well as mushrooms, smuts, wood-rot fungi, and actinomycetes. Most patients tested positive for a single species. Ando et al. (1990) even showed that the responses were serotype specific. Thus, the sensitisation seems to be specific although widely different species are involved.

Challenge studies of asthmatic cases are summarised in Table 12. Reports describing a single case identified *Neurospora* sp. and *Serpula lacrymans* as causal agents of asthma in a plywood factory worker (Côté et al., 1991) and in a patient from a home with extensive dry rot (O'Brien et al., 1978), respectively. The latter patient was also diagnosed with hypersensitivity pneumonitis.

Only four occupational studies of asthmatic patients have been found. In two of these studies specific IgE or skin prick tests were negative (Davis *et al., 1988; Seuri et al.,* 2000), indicating that the asthma was nonallergic. In three studies of hypersensitivity pneumonitis patients, obstructive responses were also observed (Pepys & Jenkins, 1968; Edwards & Davies, 1981; Greene et al., 1981) and one patient in the study by Edwards and Davies (1981) developed an immediate asthmatic response. These studies indicate that fungi and actinomycetes may also induce asthma attacks.

Two studies of nasal hypersensitivity were found. Thirteen furniture workers with positive skin prick tests to fungi and 3 with elevated total IgE from a population of 268 workers from five factories were challenged by nasal provocation with fungal extracts. Five workers had positive tests but fungal species were not reported (Wilhelmsson *et al.*, 1985). Seuri et al. (2000) tested all 14 employees from a small hospital with repeated water damage. Total fungal levels exceeded 500 cfu/m^3 at several locations, and approximately half of the counts were of *Sporobolomyces salmonicolor*. Nasal provocations with *S. salmonicolor* extract were positive in 11 workers, but skin prick tests with *S. salmonicolor* were negative in all 12 employees tested. Three of the employees were atopic as evaluated by skin prick tests with respiratory allergens.

No studies of ODTS patients challenged by bronchial provocation tests have been found, probably because of the benign course of the disease (Section 8.3).

The asthma and nasal hypersensitivity studies show that the sensitisation to fungi was IgE-mediated only in a fraction of the cases and in hypersensitivity pneumonitis patients IgE is usually absent. It seems therefore likely that other mechanisms are involved. The specificity of the response is most clearly demonstrated in hypersensitivity pneumonitis studies, where patients often were challenged with extracts of several species.

Most challenge studies used extracts. Aerosols of extracts differ from aerosols of spores by metabolite composition, particle size, and solubility. In spite of this, typical attacks of hypersensitivity pneumonitis and asthma were observed after provocation with extracts.

11.2.2. Epidemiological studies

Few studies have been found on IgE-mediated sensitisation to fungi.

Thomas et al. (1991) found no elevation of IgE against fungal species isolated from the work environment in 19 workers with work-related symptoms (cough, wheeze, or dyspnoea) selected from a population of 197 coffee workers exposed to 3×10^3 to 2×10^4 fungal cfu/m^3. IgE levels to green coffee beans and castor beans were elevated, 14% and 15%, respectively.

Zhang et al. (2005) reported high prevalences of IgE and positive skin prick tests to *Aspergillus fumigatus*, 27% and 19%, respectively, and to *Rhizopus nigricans*, 52% and 24%, respectively, in 130 Chinese tobacco workers. Fungi were measured by sedimentation on culture plates showing that these fungi were also abundant in the air.

Studies by Larsen et al. (1997) and Meyer et al. (1998) on staff members from a school contaminated by fungi failed to demonstrate IgE against species found on building construction materials by skin prick tests or Magic Lite tests in staff members. However, serum IgE against fungal species isolated from the school building could be demonstrated by histamine-release tests using blood basophils isolated from the participants (Larsen et al., 1997) or by passive immunisation with the participants serum (Meyer et al., 1998; Lander et al., 2001). No fungal levels were reported.

In 39 greenhouse workers, a high rate of sensitisation to fungi (18%) and flowers (21%) was found by skin prick testing (Monsó et al., 2002). The workers were exposed to 5×10^3 fungal cfu/m^3.

Rydjord et al. (2007) found that the IgE levels against *Rhizopus microsporus* were elevated in only 1 of 343 sawmill workers. The most highly exposed workers in this population

were wood trimmers. They were exposed to a median level of 3×10^6 spores/m^3, about half of which were from *R. microsporus* (Eduard et al., 1994). In comparsion, IgE levels against *R. microsporus* in healthy blood donor controls were elevated in 2 of 100.

The information on sensitisation to fungal spores in working populations with relatively high exposure levels is contradictory. It is surprising that sensitisation was practically absent among sawmill workers who were exposed to high levels of *Rhizopus microsporus* spores. The reason for this is not understood as tobacco workers showed a high prevalence of sensitisation to the related fungus *Rhizopus nigricans*.

The higher prevalence of sensitisation found by the histamine-release test in the indoor air studies (Larsen et al., 1997; Meyer et al., 1998) may be due to the use of whole spores of fungi isolated from the workplace, which seems more relevant than the use of fungal extracts in RASTs and skin prick tests. It is also possible that the histamine-release test is more sensitive than skin prick tests and RASTs, although good agreement has been reported for fungal allergens in children (Nolte et al., 1990; Griese et al., 1990). Both indoor air studies were performed by the same research group and confirmation of their findings in other indoor air studies and in highly exposed populations is needed (see also Section 9.2).

11.3. Effects of single exposure

Inhalation tests of naive subjects have not been performed because of concerns of inducing irreversible sensitisation and allergy.

Brinton et al. (1987) described an incident among novice college students. The students participated in a "hay party" in a poorly ventilated room where the floor had been covered with straw that emitted dense clouds of dust that did not settle rapidly. Fifty-five of 67 students developed typical ODTS symptoms with cough, muscle aches, and fever. Although no microbiological investigations were performed, it seems likely that the straw emitted large numbers of spores from fungi and/or actinomycetes. As the incident occurred in students, previous exposure to such levels is unlikely except for those raised on a farm. In any case, the number of students raised on a farm will be low, so these findings indicate that prior sensitisation is not needed for development of attacks of ODTS.

11.4. Effects of short-term exposure

This section includes provocation tests of patients with respiratory disease inhaling spores of organisms suspected to cause the disease, and epidemiological studies of acute responses recorded concurrent with exposure measurements. Both study designs may demonstrate effects of short-term exposure in repeatedly exposed individuals, and reflect exacerbations of preexisting health conditions.

11.4.1. Provocation tests

Bronchial and nasal provocation tests have been carried out to study the role of fungi and actinomycetes in hypersensitivity pneumonitis, allergic asthma, and allergic rhinitis (Section 11.2.1). In these tests, subjects are exposed to either a single dose or increasing doses of an agent until a reaction of sufficient intensity occurs or the maximum predefined dose has been reached.

Studies that have applied challenge with spores are summarised in Tables 11 and 12, and are described in more detail in Appendix 8 of Eduard (2007). Bronchial provocations with *Aspergillus clavatus, Lentinus edodes,* and *Pleurotus ostreatus* were positive in malt and mushroom workers with hypersensitivity pneumonitis after inhaled doses of 10^9, 3×10^7, and 10^7 spores, respectively. The dose of *Aspergillus clavatus* was specified as 20 mg of dry spores without further details, whereas the latter two doses were based on occupational exposure levels causing attacks of hypersensitivity pneumonitis (Riddle et al., 1968; Cox et al., 1988; Cox et al., 1989). Only a single dose was applied in these studies.

One study was performed in patients with mild nonoccupational asthma using increasing doses of spores of *Alternaria alternata* and a *Penicillium* sp. Seven patients with strongly positive skin prick tests to these fungi were challenged with graded doses (10-fold increments) of the organism they were allergic to. The spores were mixed with lactose and inhaled with Spinhalers, probably during one or a few inhalations (number not specified). The minimum doses for a positive bronchial provocation test were 9×10^4 *A. alternata* spores and $6{\cdot}10^4$ *Penicillium* sp. spores (Licorish et al., 1985).

Eight employees from moisture-damaged schools with building-related symptoms but without asthma were challenged with aerosols of *Penicillium chrysogenum* and *Trichoderma harzianum* at single concentrations during 6 minutes of 6×10^5 and 3.5×10^5 spores/m^3, respectively. No changes in mucosal or systemic symptoms, lung function, or leukocytes in blood were observed compared to placebo exposure. Participants had positive *P. chrysogenum* histamine-release tests for detection of specific serum IgE but *T. harzianum* tests were negative. Skin prick tests to these fungi were also negative (Meyer et al., 2005).

These findings suggest that asthma attacks may be induced at lower dose levels than hypersensitivity pneumonitis in IgE-sensitised individuals. However, the number of studies is small, the species vary, and it is not known if lower doses could have induced a positive response in the hypersensitivity pneumonitis studies as single dose levels were applied. The study on asthma patients (Licorish et al., 1985) indicates LOELs in a sensitive group of 2×10^4 *A. alternata* spores/m^3 and 1×10^4 *Penicillium* sp. spores/m^3 calculated to equal 8-hour exposures by dividing the applied doses with 5 m^3, assuming a minute ventilation of 10 L/min during 8 hours. The findings in the employees from moisture-damaged schools with building-related symptoms indicate NOELs of 8×10^3 *P. chrysogenum* spores/m^3 and 4×10^3 *T. harzianum* spores/m^3 calculated to equal 8-hour exposures in individuals with IgE to *P. chrysogenum* but not to *T. harzianum*. However, the significance of the detection of IgE by

the histamine-release test used in the latter study (Meyer et al., 2005) is uncertain (Section 9.2).

In one study, symptom-free garbage recycling workers and retired garbage workers with occupational asthma were tested by nasal provocation with a suspension of *A. fumigatus* spores containing approximately 6×10^7 spores/ml. No differences in inflammatory markers or nasal volume were observed between the two groups or when compared to sham challenge (Sigsgaard et al., 2000).

11.4.2. Epidemiological studies

Further information on short-term exposure was obtained from epidemiological studies of work-related symptoms and short-term functional changes, e.g., cross-shift change in lung function. Work-related irritation symptoms are reviewed in Section 11.1. Other outcomes are summarised in Appendices 6 and 7 of Eduard (2007) and studies with confounder adjustment have been summarised in Tables 13–15.

Highly exposed populations: Fever attacks of ODTS and hypersensitivity pneumonitis. Febrile attacks are most often due to ODTS (Section 8.3). The yearly incidence of ODTS has been estimated to 10–190 per 10 000 farmers (von Essen, 1990). Febrile attacks may also occur in farmers with hypersensitivity pneumonitis.

Malmberg et al. (1993) found that six farmers with diagnosed ODTS experienced fever attacks after exposure to 14×10^9 spores/m^3, and four farmers with hypersensitivity pneumonitis reported fever attacks after exposure to 3×10^9 spores/m^3 (arithmetic means). Another difference was that farmers with ODTS had carried out extraordinary tasks such as removal of spontaneously heated grain that lasted typically 1 hour, whereas farmers with hypersensitivity pneumonitis carried out work that they did once or twice daily with a duration of 15–30 minutes. Maximum exposure levels in 17 farmers without febrile symptoms were much lower, 1×10^8 spores/m^3 (arithmetic mean). Exposures had been measured when the work causing a fever attack was repeated. The exposure level calculated to equal an 8-hour exposure was 2×10^9 spores/m^3 in farmers with ODTS, 1×10^8 to 4×10^8 spores/m^3 in farmers with hypersensitivity pneumonitis, and 3×10^6 to 1×10^7 spores/m^3 in nonsymptomatic farmers. No species dominated and the proportion of actinomycetes ranged from <1% to 96%.

Malmberg et al. (1988) reported in another paper that the fever reactions were not attributable to endotoxins, as exposure levels were similar in farmers experiencing fever attacks and nonsymptomatics, although levels were high, 300 000 EU/m^3.[1]<FN1>

These results indicate a LOEL for fever attacks of 2×10^9 spores/m^3 in farmers with ODTS and a LOEL for individuals with hypersensitivity pneumonitis of 1×10^8 to 4×10^8 spores/m^3.

Highly exposed populations: Work-related cough. Work-related cough was significantly elevated (OR 3.9, 95% CI 1.0–15) in 89 farmers after exposure to 5×10^5 to 2×10^7 fungal spores/m^3 calculated to equal an 8-hour exposure and adjusted for confounders except atopy. Exposure to many other agents was measured but none were associated with cough (Eduard et al., 2001).

In 22 municipal waste handlers, cough was associated with exposure to fungal spores but not with endotoxins and bacteria. The median exposure level of the symptomatic workers was 3×10^5 spores/m^3. Confounding by smoking and age was evaluated qualitatively (Heldal & Eduard, 2004).

In both studies, cough was associated with short-term exposure to fungal spores. Exposure was measured on the same day as symptoms were recorded, a design eliminating the between-day variability provided that the symptoms are a response to short-term exposure. In the farmer study by Eduard et al. (2001), a higher exposure level was associated with an increased prevalence of cough, approximately 3×10^6 spores/m^3 (geometric mean of the range 5×10^5 to 2×10^7 spores/m^3, calculated to equal an 8-hour exposure) than in the waste handler study where a LOEL of approximately 3×10^5 spores/m^3 was found (Heldal & Eduard, 2004). This may either be a real difference, which reveals differences in the fungal flora in the two environments, or be due to a shorter exposure time in the farmer study. However, the small sizes of the populations and the lack of exposure data at the species level prevent exploration of such hypotheses. Other bioaerosol agents such as bacteria, endotoxins, and glucans were not associated with cough. Thus, LOELs of 3×10^5 spores/m^3 are indicated in waste handlers and 3×10^6 spores /m^3 in farmers.

Highly exposed populations: Lower airway inflammation. Heldal et al. (2003b) studied 25 organic waste collectors and measured inflammatory cells and markers in induced sputum on Monday morning and Thursday morning before work. Inflammatory changes were observed during this period but no correlations were found with fungal spore exposure (median 2×10^5 spores/m^3). The IL-8 increase was correlated with exposure to glucans, but glucan exposure was only weakly associated with fungal spores. This association is therefore more likely due to glucans from nonfungal origin.

Highly exposed populations: Lung function. Lung function and fungal exposure was studied during 4 workdays in 29 wood trimmers from two sawmills with median exposures of 1×10^4 and 3×10^5 cfu/m^3, respectively. The FVC decline was significant in the sawmill workers with highest exposure. FVC decline as well as forced expiratory volume in 1 second (FEV_1) and FVC as % of predicted at the end of the 4-day period were correlated with exposure to fungi. Lung function had also been measured 3 months earlier but no exposure measurements were performed at that time. Declines in FVC and FEV_1 were observed after 3 months in workers from the sawmill with the highest exposure, whereas the lung function in workers from the other sawmill did not change (Hedenstierna et al., 1986). No information on production changes was provided. It seems likely, however, that the exposure levels were similar during the follow-up as it takes time to implement preventive measures and to sort all unsorted dry timber in store.

FEV_1 and maximum expiratory flow at 25% of FVC (MEF_{25}) declined during the workweek in 28 wood trimmers and correlated with exposure to fungi (median 1×10^5 spores/m^3 dominated by *Rhizopus* and *Paecilomyces*) (Dahlquist et al., 1992). Exposure to dust, terpenes, bacteria, and endotoxins were also measured and were low except to bacteria (medians 0.3 mg/m^3, 10 ppm, 1×10^5 cells/m^3, and 20 EU/m^3, respectively).

No adjustments were made for confounders in neither study, but studying intraindividual changes in lung function reduces confounding by gender and age since subjects are compared with themselves. The effect of smoking was evaluated by separate analysis of smokers and nonsmokers, but smoking had no major influence on the associations. In the study by Dahlquist et al. (1992), exposure to total dust, endotoxins, and terpenes was low and not likely to cause lung function changes. Microscopical counts of bacteria were similar to fungal counts but were not evaluated.

Both studies showed associations between lung function changes and exposure to fungi, which was most consistent for FEV_1. The study by Dahlquist et al. (1992) indicates that associations with lung function are observable at median exposure levels of 1×10^5 spores/m^3. A more than 10 times higher level is indicated by the study by Hedenstierna et al. (1986), however.

Highly exposed populations: Upper airway inflammation. Nasal lavage fluid was analysed for inflammatory markers in two studies.

Roponen et al. (2002) studied 11 sawmill workers and found no differences between NO, TNFα, IL-4, IL-5, and IL-6 levels in nasal lavage after work compared to samples collected during vacation. The exposure level of fungal spores was 6×10^5 spores/m^3 (median, mainly *Rhizopus* and *Penicillium*) and exposure to endotoxins and terpenes was also measured.

Heldal *et al.* (2003a) performed nasal lavage in 31 municipal waste handlers on Monday morning and Thursday morning. Exposure to fungal spores (median 2×10^5 spores/m^3), bacteria, actinomycetes spores, endotoxins, and glucans was measured between the lavages. The increase in proportion of neutrophils from Monday to Thursday and the level on Thursday was associated with exposure to fungal spores, whereas other inflammatory cells, myeloperoxidase, IL-8, and eosinophilic cationic protein (ECP) were not. Possible confounding by smoking and age was evaluated qualitatively.

Both studies agree in not finding associations between fungal spore exposure and inflammatory cytokines. Heldal et al. (2003a) found associations with neutrophilic lymphocytes, which indicate an inflammatory response. Roponen et al. (2002) did not study inflammatory cells. Thus, there is some evidence for a cellular response to fungal spores at a level of 2×10^5 spores/m^3. No changes in the level of inflammatory mediators were found, however. Associations with IL-8 were expected because neutrophils are attracted by this cytokine. Possible explanations are high intra- and interindividual variability and different temporal developments of the IL-8 and neutrophil responses. A LOEL of 2×10^5 spores/m^3 for neutrophilic infiltration is thus indicated.

Highly exposed populations: Nasal congestion. Heldal et al. (2003a) studied nasal congestion in 31 municipal waste handlers by acoustic rhinometry. A correlation was found between change in nasal congestion and fungal spore exposure (median 2×10^5 spores/m^3). The association was not due to confounding or exposure to other agents (bacteria, actinomycetes spores, endotoxins, and glucans). A LOEL of 2×10^5 spores/m^3 is thus indicated by this study.

Populations exposed to common indoor air. Roponen et al. (2003) studied 41 randomly selected teachers. Exposure to fungi and bacteria were measured at work and at home. Irritation, nonspecific symptoms and the levels of NO, IL-4, IL-6, and TNFα in nasal lavage were similar in groups with high and low exposure to fungi (medians 12 and 67 cfu/m^3). IL-1β was elevated in the high-exposure group but not significantly. Particles were resuspended from the filters and tested for cytokine production with a mouse RAW264.7 macrophage cell line. In this assay, samples of the high-exposure group showed stronger IL-1β and IL-6 responses than samples of the low-exposure group, whereas no differences in TNFα and cell viability were observed.

These results are not likely to be due to random variation because the intraindividual variability of the cytokines in the nasal lavages and the mouse macrophage tests was relatively low, subjects were randomly selected, and exposure at home was included in the exposure assessment. Separate analyses in smoking and asthma status subgroups showed that the findings were not due to confounding. This study thus indicates that the two groups were exposed to particles with different inflammatory potential, but that this did not result in observable changes in nasal lavage. This may be due to differences between the individual responses as the correlation between IL-1β, IL-6, and TNFα levels in the nasal lavages and the macrophage assays were low. However, inflammatory reactions of cells in the human nose may be different from isolated cells of a mouse macrophage cell line. Furthermore, the exposure levels may have been too low for a response. The median exposure of the highest exposed group, 67 cfu/m^3, is regarded as a NOEL for irritation and nonspecific symptoms, and inflammatory markers in the upper airways. This estimate represents a 24-hour time-weighted average.

11.4.3. Discussion of effects of short-term exposure

Inhaled doses inducing a 35% increase in airway conductance in asthmatic patients after bronchial challenge were 6×10^4 *Penicillium* sp. spores and 9×10^4 *Alternaria alternata* spores (Licorish et al., 1985). The patients were sensitised to the fungus used in the challenge and thus represent a susceptible group. The dose was inhaled over a short time period. When the minimum dose for a positive challenge response was calculated to equal an 8-hour exposure by dividing the applied dose with 5 m^3 assuming a minute ventilation of 10 L/min during 8 hours, concentrations of 1×10^4

and 2×10^4 spores/m^3 were found for the two fungal species, respectively.

In employees working in moisture-damaged schools with building-related symptoms and sensitised to *P. chrysogenum* but without asthma, NOELs of 6×10^5 *P. chrysogenum* spores/m^3 and $3 \times 5{\cdot}10^5$ *T. harzianum* spores/m^3 were found by bronchial challenge to a single concentration for 6 minutes (Meyer et al., 2005). Also this group may be susceptible although less than the former. The applied doses are equal to 4×10^3 to 8×10^3 spores/m^3 for 8 hours.

In highly exposed working populations (farmers, sawmill workers, and waste handlers), LOELs of 1×10^5 to 2×10^5 spores/m^3 were found for nasal congestion, eye irritation, sore throat, and lung function changes (Hedenstierna et al., 1986; Dahlquist et al., 1992; Alwis et al., 1999; Eduard et al., 2001; Heldal et al., 2003a). A higher LOEL of 3×10^6 spores/m^3 was found for nose irritation in farmers, but the prevalence was already increased (OR 4.1, 95% CI 0.88–19) at an exposure level of 1×10^5 spores/m^3, although not significantly (Eduard et al., 2001). No changes in inflammatory markers were found in the upper or lower airways in four studies of sawmill workers, waste handlers, and teachers (Roponen et al., 2002, 2003; Heldal et al., 2003a, 2003b). However, in waste handlers, neutrophils in nasal lavage but not in induced sputum were associated with fungal spore exposure at a median level of 2×10^5 spores/m^3 (Heldal et al., 2003a).

Cough seems to be related to higher exposure levels with LOELs of 3×10^5 spores/m^3 in a waste handler study (Heldal & Eduard, 2004) and 3×10^6 spores/m^3 in a study of farmers (Eduard et al., 2001).

Even higher levels induced fever. Bronchial challenge of patients with hypersensitivity pneumonitis using the main species found in the environment *(Aspergillus clavatus, Pleurotus ostreatus,* and *Lentinus edodes)* induced typical attacks, including fever, in mushroom workers (Cox et al., 1988; Cox et al., 1989) and a malt worker (Riddle et al., 1968). The applied doses are equal to 2×10^6 to 2×10^7 spores/m^3 (calculated to equal an 8-hour exposure). An epidemiological study in farmers (Malmberg et al., 1993) indicated even higher exposure levels, 1×10^8 to 4×10^8 spores/m^3 in hypersensitivity pneumonitis patients, 2×10^9 spores/m^3 in ODTS patients, and 3×10^6 to 1×10^7 spores/m^3 in nonsymptomatic farmers (equal to an 8-hour exposure). The farmers were exposed to spores from various fungal and actinomycete species.

The response in bronchial challenge studies of patients with hypersensitivity pneumonitis or asthma shows species specificity (Section 11.2.1), whereas attacks of ODTS seem not to require previous sensitisation (Section 8.3). The other studies provided little information on the importance of specific fungi.

11.5. Effects of long-term exposure

11.5.1. Epidemiological studies

Epidemiological studies of hypersensitivity pneumonitis, asthma, chronic bronchitis, and systemic symptoms are reviewed as well as studies of chronic lung function loss and X-ray changes.

Highly exposed populations: Hypersensitivity pneumonitis. The role of fungal spores in occupational respiratory disease was probably first recognised in hypersensitivity pneumonitis (Section 8.2). The incidence of hypersensitivity pneumonitis is lower than that of ODTS, 2–30, and 10–190 per 10,000 farmers/year, respectively (von Essen, 1990).

Ávila and Lacey (1974) diagnosed 26 patients with hypersensitivity pneumonitis among 648 cork workers in a cross-sectional study (prevalence 4%). *Penicillium glabrum* (previous name *frequentans*) was the dominating fungus, with exposure levels ranging from 1×10^6 to 7×10^7 spores/m^3 in different departments. Current exposure was not associated with hypersensitivity pneumonitis. However, as the disease develops after prolonged exposure and workers changed jobs frequently, current exposure was not a good measure of relevant exposure. The role of *P. glabrum* was demonstrated by bronchial provocation of 19 cork workers (Table 11). Although information on historical exposure levels is lacking, these may not be very different from current exposure levels as no mention was made of preventive measures in spite of the large number of workers with hypersensitivity pneumonitis. It seems therefore possible that hypersensitivity pneumonitis can develop after prolonged exposure to the mean levels found in this study, approximately 10^7 spores/m^3.

Wenzel and Emanuel (1967) found five cases of hypersensitivity pneumonitis with interstitial pneumonitis and granuloma among 37 papermill workers (prevalence 14%) exposed to spores of *Cryptostroma corticale*. Cases typically developed during periods when exposure was highest (mean level probably 5×10^6 *C. corticale* spores/m^3). There is uncertainty about this level, however, because stationary sampling was applied and the presented data did not describe the measurement unit.

Both studies suggest that prolonged exposure to approximately 10^7 spores/m^3 of *P. glabrum* and *C. corticale* can induce hypersensitivity pneumonitis, but the exposure estimates are crude since exposure was poorly documented. Results were not adjusted for confounders either.

Highly exposed populations: Asthma. Current physician diagnosed asthma was studied in a cross-sectional study of 1614 farmers. Annual exposure levels to fungal spores (1 year equalled geometric mean 2×10^6 spores/m^3), bacteria, endotoxins, and ammonia were estimated (Eduard et al., 2004). Exposure to fungi was positively correlated with asthma in the highest exposed (4×10^6 to 4×10^7 spores/m^3) nonatopic farmers with OR 1.7 (95% CI 1.0–2.7). In atopic farmers, exposure to fungi was negatively correlated with asthma with OR 0.28 (95% CI 0.10–0.78). The associations with fungal spores were stronger than with endotoxins and of similar strength as the associations with ammonia. Associations were adjusted for age, smoking, gender, and asthma in the family. Year-average exposure levels were estimated because it was not clear when asthma was initiated and occupational asthma may take years of exposure

to develop. Atopic status was important as the association was reversed in atopic farmers. Endotoxins and amonia may also showed similar associations but that may be due to correlated exposure levels. Moreover, it seems not biologically plausible that ammonia is the cause of asthma because the annual exposure level was 4 ppm.

In three studies, indicators of asthma were investigated (Coenen et al., 1997; Alwis et al., 1999; Monsó et al., 2002). Variability of peak expiratory flow was studied in 72 waste collectors and exposure was estimated by a job exposure matrix including total fungi (approximate level 2×10^5 cfu/m^3), bacteria, and endotoxins (Coenen et al., 1997). Peak flow variability was significantly increased in the group with the highest exposure to *A. fumigatus* (approximate level 10^4 cfu/m^3), but was not associated with any other agent.

Wheezing was studied in 82 joinery and 108 sawmill and chip mill workers (Alwis et al., 1999). Exposure to fungal spores (arithmetic mean 3×10^4 cfu/m^3), dust, Gram-negative bacteria, endotoxins, and glucans was measured. Wheezing was associated with fungal exposure (OR 1.4, 95% CI 1.0–2.0) but more strongly with respirable glucans. The association between wheezing and fungal exposure may be due to glucan exposure because these exposures were strongly correlated ($r = .76$–$.86$).

Monsó et al. (2002) studied wheeze and asthma attacks in 39 greenhouse workers and measured exposure to endotoxins, bacteria, and fungi (median 5×10^3 cfu/m^3). No associations were found but the number of workers with symptoms was small, especially of asthma attacks ($n = 3$).

Current exposure is probably a valid exposure estimate for peak flow variability and wheezing, because these outcomes are likely to be a response to recent exposure. In the first two studies, the role of other bioaerosol components such as bacteria and endotoxins was also evaluated. The waste collector study (Coenen et al., 1997) indicated that peak flow variability was a specific response to *A. fumigatus* but associations were not adjusted for confounders. The greenhouse worker study (Monsó et al., 2002) was small and included only three subjects with asthma attacks. The results of the wood worker study (Alwis et al., 1999) are therefore the most reliable. In that study, wheezing was more strongly associated with glucans than fungi. This may, however, indicate a response to a fungal component as glucans and fungi were correlated.

In summary, wheezing was associated with fungal exposure in wood workers exposed to approximately 3×10^5 spores/m^3 (arithmetic mean) (Alwis et al., 1999). This level is lower than indicated by the increased prevalence of asthma in nonatopic farmers, approximately 1×10^7 spores/m^3 (geometric mean of the range) (Eduard et al., 2004). This might indicate that nonatopic asthma is induced by higher exposure levels than wheezing. However, atopy was not evaluated in the wood worker study and the association with wheezing might be due to the atopic individuals in the population. In the farmer study, atopic individuals had been excluded in the analysis of nonatopic asthma and this association was therefore not confounded by atopy. Furthermore, the exposure in the asthma study among farmers was estimated with considerable random error, which may have attenuated exposure-response associations. Wheezing is not specific to asthma, as the prevalence of wheezing is much higher than that of asthma. The negative association with atopic asthma in farmers is special (Eduard et al., 2004). This may be due to a protective effect, but may also be explained by selection, either by retirement or by changing production. There is uncertainty about the role of fungi because levels of other bioaerosol agents that were associated with these outcomes were also correlated with fungal levels.

*Highly exposed populations: Chronic bronchitis.*Chronic bronchitis and productive cough were studied in 72 waste collectors. Symptoms were monitored during 2 weeks, and exposure to bacteria, endotoxins, and culturable fungi was estimated by a quantitative job exposure matrix. Both outcomes were significantly increased in the group with highest exposure to *A. fumigatus* (exposure level approximately 10^4 cfu/m^3), but these outcomes were not associated with total fungi (approximate exposure level 2×10^5 cfu/m^3). The results were not adjusted for confounders, however (Coenen et al., 1997).

Chronic bronchitis was also studied by a general questionnaire in 951 waste collectors and 423 park workers (Hansen et al., 1997). Exposure of waste collectors to culturable and total fungi, inorganic dust, organic dust, irritant gases, and fumes was estimated by a quantitative job exposure matrix. The waste collectors were categorised in two groups; those exposed to low ($<2 \times 10^5$ cfu/m^3) and high ($\geq 2 \times 10^5$ cfu/m^3) levels, respectively. The groups were of very different sizes (at the 96th percentile) without justification, and no other exposure data were presented. The exposure of the park workers was not measured and assumed to be low. Chronic bronchitis was significantly elevated in the low-exposure group (prevalence proportion ratio (PPR) 1.9, 95% CI 1.0–3.6) for both culturable fungi and fungal spore count. The PPR increased further in the high-exposure group to 2.5 (95% CI 0.6–11) for countable fungi and 2.7 (95% CI 0.7–11) for culturable fungi, but not significantly.

Alwis et al. (1999) studied bronchitis, phlegm, and breathlessness in 82 joinery workers, and 108 sawmill and chip mill workers. Breathlessness (OR 4.8, 95% CI 1.4–17) was most strongly associated with fungal exposure in sawmill and chip mill workers (arithmetic mean 4×10^4 cfu/m^3). Phlegm (OR 2.0, 95% CI 1.0–3.9) was also associated with fungal exposure in joinery workers (arithmetic mean 1×10^4 cfu/m^3). Endotoxin exposure was also associated with phlegm, but the association was slightly weaker. Confounding of the association with fungal exposure is not likely because endotoxin exposure was only moderately correlated with fungal exposure. Exposure to fungi was also associated with bronchitis in joinery workers (OR 3.0, 95% CI 1.3–6.6, arithmetic mean 1×10^4 cfu/m^3) and sawmill/chip mill workers (OR 2.9, 95% CI 1.1–7.2, arithmetic mean 4×10^4 cfu/m^3). The associations with glucans were stronger (OR 20, 95% CI 10–41), however. The association between bronchitis and fungal exposure may be due to glucan exposure because these exposures

were strongly correlated ($r = .76–.86$). However, the associations with glucan exposure may also reflect fungal exposure because glucans are a structural component of fungi.

Morning cough was associated with exposure to fungal spores in 107 wood trimmers (Eduard et al., 1994). Exposure to different spore types (42% were from *Rhizopus microsporus)*, pine dust, and spruce dust was measured. Work-related chest tightness was associated with *Rhizopus microsporus* spore and wood dust exposure, but this association may suffer from reporting bias because the symptom had been recorded conditional on handling of mouldy timber. Exposure levels of 2×10^6 spores/m^3 predicted an OR of 1.5 for both associations. The study did not include an external reference group. Therefore, the lowest exposed wood trimmers (4×10^5 spores/m^3) were used as an internal reference group.

To sum up, symptoms suggestive of chronic bronchitis were associated with fungal exposure in five studies performed on three different occupations. Two studies of waste collectors specifically reported chronic bronchitis. In one of these, no association was found with total fungi (approximate exposure level 10^4 cfu/m^3), although associations were not adjusted for confounders (Coenen et al., 1997). An association was reported in the other study but the expsure level is unclear (Hansen et al., 1997). A LOEL of 2×10^6 spores/m^3 was indicated for morning cough in the wood trimmer study (Eduard et al., 1994), but this is a high estimate because the lowest exposed group was exposed to 4×10^5 spores/m^3 and lower exposure levels could thus not be explored.

The associations with breathlessness and phlegm reported by Alwis et al. (1999) and morning cough by Eduard et al. (1994) in wood workers seem reliable because the exposure of wood workers is mainly to wood dust and fungal spores and the study by Eduard et al. (1994) also adjusted for wood dust exposure. It is therefore likely that the association between chronic bronchitis symptoms and exposure to fungal spores is real. LOELs of 1×10^5 and 4×10^5 spores/m^3 were indicated for phlegm in joinery workers and for breathlessness in sawmill and chip mill workers, respectively. In wood trimmers, a LOEL of 2×10^6 spores/m^3 was found for morning cough, but this is probably a high estimate because the internal reference group in this population was relatively highly exposed.

In two studies, stronger associations between bronchitis symptoms and specific fungi were found than with total fungi; *A. fumigatus* in waste collectors and *R. microsporus* spores in wood trimmers (Eduard et al., 1994; Coenen et al., 1997). However, both studies suffer from methodological weaknesses.

Highly exposed populations: Lung function. Reduced restrictive lung function (described as a "restrictive effect" but not further documented) was observed in 648 cork workers (Ávila & Lacey, 1974). *Penicillium glabrum* was the dominating fungus and total spore levels ranged from 1×10^6 to 7×10^7 spores/m^3 in different departments. Restrictive lung function was not associated with spore exposure but increased from 8% in workers employed for up to 5 years to 29% after more than 30 years of employment. Impaired lung function is expected to be associated with long-term exposure as it may take years before the decline is sufficiently large to be detectable. Duration of employment is probably a reasonable estimate of cumulative exposure because workers changed jobs frequently. As it seems possible that current exposure levels were not very different from the past (see "Highly exposed populations: Hypersensitivity pneumonitis" in Section 11.5.1), these results indicate that long-term exposure to approximately 10^7 spores/m^3 induced restrictive lung function impairment.

Johard et al. (1992) compared 19 wood trimmers exposed to fungi (median 1×10^5 spores/m^3) with 19 not occupationally exposed subjects, all nonsmokers. Lung function (vital capacity, FEV_1, diffusion capacity for CO) was not significantly different compared to the controls. These results suggest a NOEL of 1×10^5 spores/m^3 for long-term decline in FVC and FEV_1 decline. However, in another publication of this study, an association was found between fungal spore exposure and short-term decline in FEV_1 and MEF_{25} (Dahlquist et al., 1992).

Highly exposed populations: Systemic symptoms. Ivens *et al.* (1999) studied diarrhoea in 950 waste collectors, with 387 outdoor municipal workers as reference. Inhaled doses of culturable fungi, total fungi, and endotoxins were estimated by a quantitative job exposure matrix for the waste collectors but not the reference group. The waste handlers were categorised in three exposure groups of very different sizes. Diarrhoea was significantly elevated in the lowest exposed waste collectors (PPR 3.0, 95% CI 1.9–4.9) for fungal spores, and increased in a dose-response manner in the more highly exposed groups. Adjustments for confounders were applied but not for other agents, although similar associations were observed for endotoxins.

No other studies of systemic symptoms have been found.

Highly exposed populations: X-ray changes. Wenzel and Emanuel (1967) observed diffuse reticulo-nodular infiltrates by chest X-ray in 40% of 37 papermill workers exposed to spores of *Cryptostroma corticale.* These changes were observed during summer and winter, and the mean exposure was approximately 5×10^6 *C. corticale* spores/m^3. There is, however, uncertainty about these levels (see "Highly exposed populations: Hypersensitivity pneumonitis" in Section 11.5.1 and Appendix 6 of Eduard [2007]).

Reticulo-nodular changes were observed by chest X-ray in 57% of 648 cork workers (Ávila & Lacey, 1974). *Penicillium glabrum* was the dominating fungus and total spore levels ranged from 1×10^6 to 7×10^7 spores/m^3 in different departments. The X-ray changes correlated with current exposure to fungal spores in nonsmokers but not with duration of employment.

Both studies indicate that X-ray changes occur in populations exposed to fungal spores and wood or cork dust. Although confounding was not adjusted for in the papermill study, and only smoking was considered in the cork worker study, it seems likely that fungal spores may induce these X-ray changes since the prevalence of this outcome was very high. Wood or cork dust is not known

to induce X-ray changes, whereas this is a common finding in hypersensitivity pneumonitis. The cork worker study (Ávila & Lacey, 1974) suggests that exposure levels of 10^7 *Penicillium glabrum* spores/m^3 can induce the X-ray changes, and as cork workers changed jobs regularly, the correlation with current exposure levels indicates that the X-ray changes do not take very long time to develop. A similar value is indicated in the papermill study (Wenzel & Emanuel, 1967), but there is uncertainty about the unit of exposure in this study.

Highly exposed populations: Lower airway inflammation. Johard et al. (1992) studied 19 wood trimmers exposed to approximately 1×10^5 spores/m^3 and to 10^5 bacteria/m^3, and 25 subjects not occupationally exposed to fungi, by bronchoalveolar lavage. Cell counts were not different, but albumin, fibronectin, and hyaluronan were significantly higher in the sawmill workers than in the controls, indicating low-intensity alveolar inflammation. Results were not adjusted for confounders but all participants were nonsmokers. Exposures to total dust, endotoxins, and terpenes were reported to be low in another publication of this study, with medians of 0.3 mg/m^3, 20 EU/m^3, and 10 ppm, respectively (Dahlquist et al., 1992).

Populations exposed to common indoor air: Respiratory symptoms. Li et al. (1997) studied cough and phlegm in 264 day-care workers. Fungi were measured in indoor and outdoor air (geometric means 1200 and 1000 cfu/m^3, respectively), and house dust mite allergens in settled dust. *Cladosporium*, *Penicillium, Aspergillus,* and yeasts were prevalent species. Cough and phlegm were associated with *Aspergillus* levels (geometric mean 32 cfu/m^3) but not with other species or total fungi.

Menzies et al. (1998) compared 107 office workers with work-related respiratory symptoms and 107 office workers without symptoms. Airborne fungi (mean *Alternaria* levels 1 and 0.3 cfu/m^3, respectively, total fungal levels not reported) were measured as well as fungi and house dust mite allergens in floor dust. Symptoms were associated with *Alternaria* exposure (OR 4.2, 95% CI 1.1–16).

Purokivi et al. (2001) compared 37 employees from a school with fungal problems with 23 employees from a school without such problems. Culturable fungi were measured during the winter (geometric mean 29 and 6 cfu/m^3, respectively). Respiratory symptom prevalences were not significantly different at the end of spring, but no confounder adjustments were carried out.

Roponen et al. (2003) recorded symptoms of the lower airways in randomly selected teachers, and exposure to fungi and bacteria was measured at home and at school. The population was divided in a low- and a high-exposure group (median 12 and 67 cfu/m^3, respectively). Lower airways symptoms were similar in the two groups.

No study found associations between respiratory symptoms and total fungi. The study by Li et al. (1997) is interesting because they used random selection and controlled for confounders, which strengthen the reliability of their results. However, it is not clear how fungal exposure was measured. Fungal levels in this study were considerable higher than in the other studies, 1 200 cfu/m^3, which is 20–500 times higher than in the studies by Purokivi et al. (2001) and Roponen et al. (2003). Menzies et al. (1998) only quoted *Alternaria* levels, which were very low. The study by Li et al. (1997) suggests a NOEL of approximately 10^4 spores/m^3, and the study by Roponen et al. (2003) a NOEL of approximately 7×10^2 spores/m^3 for a 24-hour exposure.

Findings in the studies by Li et al. (1997) and Menzies et al. (1998) suggest that *Aspergillus* and *Alternaria* are more potent than other species as they were associated with symptoms but were not the dominating species.

Populations exposed to common indoor air: Systemic symptoms. Two studies described in the previous section also studied systemic symptoms.

Li et al. (1997) studied lethargy and fatigue in day-care workers and found associations with *Aspergillus* levels (geometric mean 32 cfu/m^3) but not with total culturable fungal levels (geometric mean 1200 cfu/m^3).

Roponen et al. (2003) found that nonspecific symptoms in randomly selected teachers were not different in individuals with low and high exposure (median 12 and 67 cfu/m^3, respectively), including exposure both at work and home.

The day-care workers were exposed to the highest levels but associations were only found for *Aspergillus*, which constituted only a small fraction of the fungal exposure. This might indicate that *Aspergillus* species are more potent than other fungi. However, nonspecific symptoms are commonly associated with dampness in buildings, but a specific role of fungi has not yet been demonstrated; review by Bornehag et al. (2001). It can therefore not be ruled out that *Aspergillus* sp. is just a marker of humidity in buildings.

Populations exposed to common indoor air: Airway inflammation. Hirvonen et al. (1999) studied 32 school staff members of a school contaminated with fungi and 8 or 25 healthy controls from a research institution without known fungal contamination (the numbers of controls were inconsistent). Increased levels of NO, TNFα, and IL-6 were observed in nasal lavage after the spring and/or autumn term compared to after vacation. These levels were also higher than in external controls. Fungal levels in the school in the autumn were 7–100 cfu/m^3. However, no exposure data after vacation or of the control subjects were reported.

Purokivi et al. (2001) compared nasal lavage and induced sputum samples of school employees from schools with and without fungal problems. Culturable fungi were only measured during the winter (geometric mean 29 and 6 cfu/m^3, respectively). Higher levels of IL-1 and IL-4 were found in nasal lavage and of IL-6 in induced sputum at the end of the spring term in employees from the school with fungal problems. Differential cell counts were similar. No confounder adjustments were carried out, however.

Thus, both studies found increased cytokine levels in nasal lavage and/or induced sputum after school terms. These findings cannot be related to exposure, however, due to lack of exposure data.

Populations exposed to common indoor air: Asthma and rhinitis. Seuri *et al.* (2000) described a cluster of 4 asthma (6 tested) and 11 rhinitis cases in 14 employees from a hospital with water damage. Fungal levels exceeded 500 cfu/m^3 at three locations with a maximum of 1400 cfu/m^3 (approximately 50% *Sporobolomyces salmonicolor).* The asthma and rhinitis cases had positive challenges with *S. salmonicolor* extract but skin prick tests with the extract were negative indicating that asthma and rhinitis were nonallergic (see also Section 11.2.1). Serum IgG antibodies to typical water-damage fungi and *S. salmonicolor* were found in most employees, which further demonstrated exposure to these fungi. Exposure levels had probably been higher as a water-damaged floor had been repaired a year earlier, but no microbial measurements were then carried out.

This study indicates that a high proportion of workers in a water-damaged building developed nonallergic asthma and rhinitis, and the response was specific for the fungus *S. salmonicolor,* which was also the dominating species in the building. Symptoms were maintained at exposure levels of approximately 10^4 spores/m^3, but induction of the diseases probably occurred at higher levels.

11.5.2. Discussion of effects of long-term exposure

Symptoms in employees with nonallergic asthma and rhinitis in the hospital study (Seuri et al., 2000) indicated a LOEL of 1×10^4 spores/m^3. The exposure levels inducing the diseases were probably higher. In farmers, a much higher LOEL of approximately 10^7 spores/m^3 was indicated for nonatopic asthma (Eduard et al., 2004). This association may, however, have been attenuated by considerable nondifferential misclassification of the exposure and have resulted in a high estimate of the effect level. Also, exposure to other biological agents may explain the association in the farmer study.

The negative association with asthma in atopic farmers is special (Eduard et al., 2004). It may be due to a protective effect that has been ascribed to microbial exposure (the so-called hygiene hypothesis), but it may also be due to selection. The role of other biological agents cannot be ruled out, as they were correlated with exposure to fungal spores. It is interesting, however, that none of the asthma and rhinitis cases in the hospital employee study were atopic (Seuri et al., 2000).

Bronchitis symptoms were associated with fungal exposure in five studies of three different occupations. Most populations were exposed to median levels of 1×10^4 to 4×10^4 fungal cfu/m^3, which indicate LOELs of 1×10^5 to 4×10^5 spores/m^3. Studies performed on sawmill workers, and especially wood trimmers, are of special interest since exposure to agents other than fungal spores is usually low. For respiratory symptoms such as phlegm and breathlessness in wood workers, LOELs of 1×10^5 spores/m^3 and 4×10^5 spores/m^3 were reported (Alwis et al., 1999). In wood trimmers, a LOEL of 2×10^6 spores/m^3 was found for morning cough. This is a high estimate because the population did not include individuals with low exposure (Eduard et al., 1994).

No associations were found between respiratory symptoms and exposure to "total fungi" in four common indoor air studies (Li et al., 1997; Menzies et al., 1998; Purokivi et al., 2001; Roponen et al., 2003). The exposure level in the study with the highest exposure was approximately 10^4 spores/m^3, which was measured by stationary sampling. The exposure level would probably have been higher if measured by personal sampling. Furthermore, the documentation of the measurements was poor (Li et al., 1997). This NOEL is therefore consistent with the LOELs found for respiratory symptoms in more highly exposed populations.

The wood worker study indicates a NOEL of 1×10^5 spores/m^3 for lung function changes (Johard et al., 1992). A reduced FVC observed cross-sectionally in cork workers exposed to approximately 10^7 spores/m^3 may be consistent with this NOEL (Ávila & Lacey, 1974). Results were poorly documented in both studies, however.

Exposure levels of approximately 10^7 spores/m^3 of *Penicillium glabrum* and *Cryptostroma corticale* were related to hypersensitivity pneumonitis and X-ray changes (Wenzel & Emanuel, 1967; Ávila & Lacey, 1974). These exposure levels are crude estimates because of poorly documented exposure, and results not adjusted for confounders. However, the exposure was mainly to fungal spores, and wood or cork dust and only fungal spores are known to induce these outcomes. Furthermore, the prevalence of the X-ray changes was very high and cases of hypersensitivity pneumonitis were confirmed by bronchial provocation tests. It seems therefore unlikely that confounder adjustment would have changed these associations substantially.

Inflammatory markers in bronchoalveolar lavage were increased in wood trimmers exposed to 10^5 spores/m^3, whereas inflammatory cells were not (Johard et al., 1992). In two common indoor air studies, inflammatory markers in nasal lavage and induced sputum in school personnel working in a moisture-damaged building where higher than in controls and school employees working in a nonproblem building, respectively, but the effects could not be related to fungal exposure (Hirvonen et al., 1999; Purokivi et al., 2001).

An association was reported between fungi and diarrhoea in a study of waste handlers (Ivens et al., 1999), but this was the only study that reported this outcome. Two common indoor studies did not find associations between fungal exposure and the nonspecific symptoms lethargy and fatigue (Li et al., 1997; Roponen et al., 2003).

Few studies have analysed results for specific fungi. A study of waste handlers reported associations between *A. fumigatus* and variable peak flow and bronchitis symptoms, but no confounder adjustments were applied (Coenen et al., 1997). The indicated LOEL, 1×10^5 spores/m^3, was somewhat lower than that for lung function changes and respiratory symptoms. In a wood trimmer study, work-related chest tightness was associated to *Rhizopus microspores,* with a LOEL of 2×10^6 spores/m^3, but this estimate may be biased and is a high estimate since the population did not include individuals with low exposure (Eduard et al., 1994). X-ray

changes and hypersensitivity pneumonitis were observed in wood and cork workers exposed to spores mainly from *Cryptostroma corticale* and *Penicillium glabrum*, respectively (Wenzel & Emanuel, 1967; Ávila & Lacey, 1974). Common indoor air studies showed associations between respiratory symptoms and *Aspergillus* sp. and *Alternaria* sp. (Li et al., 1997; Menzies et al., 1998), and between asthma and rhinitis and *Sporobolomyces salmonicolor* (Seuri et al., 2000). A role for *S. salmonicolor* is probable as this association was further confirmed by provocation tests with the organism. The exposure levels of the specific organisms in the other two studies were very low, however. Three studies support that specific organisms may be more toxic (Li et al., 1997; Coenen et al., 1997; Menzies et al., 1998), but these studies have important shortcomings in the exposure assessment.

11.6. Genotoxic and carcinogenic effects

No studies including fungal spore exposure have been found.

11.7. Reproductive and developmental effects

No studies including fungal spore exposure have been found.

12. Dose-effect and dose-response relationships

12.1. Dose considerations and extrapolation from animal studies

Intratracheal and intranasal instillation circumvent the normal deposition processses in the airways, and distribute spores deeper into the lung. The dose that reaches the lung is also increased compared with inhalation. However, intratracheally instilled dust was less homogeneously distributed in the lung with little dust reaching the periphery (Pritchard, 1985). This may lead to local overload conditions and Pritchard et al. recommended not to extrapolate dose-response relationships from animal studies that applied intratracheal instillation to the human condition (Pritchard, 1985). Such studies are therefore not considered further.

In some animal inhalation studies, the applied dose was estimated by viable counts in lung tissue obtained shortly (up to 4 hours) after challenge. This method probably underestimates the applied dose to a large extent and such studies were also omitted in the dose-effect and dose-response considerations.

Due to differences between the lungs of humans and laboratory animals, exposure levels applied in animal experiments should be extrapolated. These factors vary substantially from 0.03 to 7 and depend on dose metric and particle size (Jarabek et al., 2005). As the relevant dose metric is not clear, no attempts were made to specify an extrapolation factor.

Results from human challenge studies were extrapolated from the dose that was inhaled during a few minutes to the average concentration over an 8-hour work shift. This may be an underestimation as "the concentration is often more important than duration of exposure" (Jarabek et al., 2005). See also Section 10.1.

12.2. Effects related to single and short-term exposure

12.2.1. Animal studies

Only 1 out of 32 animal studies is relevant for evaluation of dose-effect and dose-response associations. In that study, Guinea pigs were exposed to airborne fungal spores for 4 hours/day, for 1 day or for 5 days/week during 3 and 5 weeks, a time frame not very different from workplace exposure (Fogelmark et al., 1991). Four fungal and one actinomycete species were tested, but only exposure to 3×10^8 spores/m^3 of *Saccharopolyspora rectivirgula* showed increased neutrophil and eosinophil counts in bronchoalveolar lavage 24 hours after a single exposure for 4 h. None of the fungal species induced a response after a single exposure. These results indicate that a single exposure to fairly high concentrations of four species, including *Aspergillus fumigatus* (approximately 3×10^7 spores/m^3 and similar or higher for the other fungi) for 4 hours do not induce inflammation in Guinea pigs. Table 16 summarises the effects observed after exposure for 3 and 5 weeks. Only one dose level was applied, except for *Aspergillus fumigatus* that was tested at two concentrations. Exposure levels were probably underestimated because spores were counted by microscopy after resuspension from membrane filters with a pore size of 0.8 µm. A fraction of the smaller spore types, e.g., from *Aspergillus* and *Penicillium* will be trapped in the filter matrix and lost in the analysis but this is not accounted for in the paper (Section 10.5).

All species induced inflammatory changes in the lung after repeated exposure. The lowest LOEL was 7×10^5 spores/m^3 for *A. fumigatus* that induced slight lung inflammation. Extrapolating from 4 to 8 hours of exposure, this level corresponds to 4×10^5 spores/m^3. It should be noted, however, that probably a smaller proportion of the inhaled spores reaches the alveolar region in rodents than in humans.

The following differences in inflammatory potency between the fungal and the actinomycete species can be deduced from these results: *A. fumigatus* was more toxic than *Rhizopus stolonifera* and *Phanerochaete chrysosporium, and Penicillium aurantiogriseum* was more toxic than *P. chrysosporium.* Exposure to 3×10^8 *S. rectivirgula* spores/m^3 induced inflammation at a similar level as exposure to 2×10^7 *R. stolonifera* spores/m^3. Further differences between fungal species have been reported in other in vivo studies. Especially mycotoxin containing spores and spores from pathogenic fungi, e.g., *A. fumigatus*, showed the highest inflammatory potential (Sections 10.4.5 and 10.9).

12.2.2. Challenge studies of symptomatic subjects

Human challenge studies with spores are summarised in Table 17. These studies indicate a LOEL above 1×10^4 to 2×10^4 spores/m^3 for *Penicillium* species and *Alternaria alternata* in healthy subjects, as this level was the lowest that induced significant airway obstruction in asthmatic patients allergic to these fungi (Licorish et al., 1985). The NOELs of 8×10^3 and 4×10^3 spores/m^3 observed for *Penicillium chrysogenum* and

Table 16. LOELs of 5 fungal species in Guinea pigs exposed to spore aerosols for 4 hours/day, 5 days/week for 3 and 5 weeks (Fogelmark et al., 1991).

Species	LOEL, spores/m^3	Effects
Aspergillus fumigatus	7×10^5	After 3 as well as 5 wk: BAL: Increased lymphocyte and eosinophil counts. Lung tissue: Slight cell infiltration of the alveoli.
Aspergillus fumigatus	3×10^7	After 3 as well as 5 wk: BAL: Increased AM, lymphocyte, neutrophil, and eosinophil counts. lung tissue: Cell aggression in the alveoli, alveolar wall thickening with interstitial cells, and granuloma formation.
Rhizopus stolonifera	2×10^7	After 3 as well as 5 wk: BAL: Increased AM, neutrophil, lymphocyte, and eosinophil counts. Lung tissue: Cell infiltration in alveoli and interstitial tissue, alveolar wall thickening.
Saccharopolyspora rectivirgula	3×10^8	After 3 as well as 5 wk: BAL: Increased AM, neutrophils, lymphocytes, and eosinophils counts. Lung tissue: Cell infiltration in alveoli and interstitial tissue, alveolar wall thickening.
Penicillium aurantiogriseum	1×10^9	After 5 wk: BAL: Increased AM, neutrophils, lymphocyte, and eosinophil counts. Lung tissue: Defined granulomas.
Phanerochaete chrysosporium	2×10^9	After 5 wk: BAL: Increased AM, neutrophil, lymphocyte, and eosinophil counts. Lung tissue: Severe cell infiltration in alveoli and interstitial tissue, alveolar wall thickening, and granuloma formation.

AM: alveolar macrophages; BAL: bronchoalveolar lavage; LOEL: lowest observed effect level.

Table 17. Effects observed in symptomatic subjects in challenge studies with whole spores.

NOEL (spores/m^3)	LOEL (spores/m^3)	Species	Study group	Effects	Comments	Reference
8×10^{3a}		*Penicillium chrysogenum*	8 school employees with sick building syndrome	No changes in mucosal symptoms, systemic symptoms, lung function and blood leukocytes.	Provocation tests compared to sham exposure of the same subjects.	Meyer et al. (2005)
4×10^{3a}		*Trichoderma harzianum*				
	1×10^{4b}	*Penicillium* sp.	3 asthma patients	Immediate and late decrease in airway conductance.	Provocation tests with increasing dose with steps of 10× and interpolation between doses to find a 35% decrease in specific airway conductance.	Licorish et al. (1985)
	2×10^{4b}	*Alternaria alternata*	4 asthma patients (occupation unknown in both groups)	Immediate and late decrease in airway conductance.		
	2×10^{6b}	*Pleurotus ostreatus*	4 mushroom workers with hypersensitivity pneumonitis	After 6–8 h, fever, chills, muscle pain, dyspnoea, leukocytosis, decreased FVC.	Provocations tests with a single dose.	Cox et al. (1988)
	6×10^{6b}	*Lentinus edodes*	5 mushroom workers with hypersensitivity pneumonitis	After 6 h, fever, chills, muscle pain, dyspnoea, leukocytosis, and decreased FVC. TLco and PaO_2 decreased in 1/5.	Provocation tests with a single dose.	Cox et al. (1989)

[a]Calculated to equal an 8-hour exposure by the weight factor 8 hours/exposure time.
[b]Calculated to equal an 8-hour exposure by dividing the applied dose with 5 m^3, assuming a minute ventilation of 10 L/min during 8 hours.
FVC: forced vital capacity; LOEL: lowest observed effect level; NOEL: no observed effect level; PaO_2: arterial oxygen tension; TLco: lung transfer factor for carbon monoxide (measure of gas diffusion).

Trichoderma harzianum, respectively, in school employees with sick building syndrome (Meyer et al., 2005) are consistent with the former study. Higher LOELs were found in mushroom workers with hypersensitivity pneumonitis (Cox et al., 1988, 1989). However, only the study by Licorish et al. (1985) applied more than one dose level so the NOELs and LOELs from the studies by Cox et al. and Meyer et al. are only crude estimates (Cox et al., 1988, 1989; Meyer et al., 2005).

12.2.3. Epidemiological studies

Epidemiological studies of effects such as short-term changes in lung function, changes in inflammatory markers in the airways, and studies where symptoms were recorded on the same day as exposure measurements were performed are considered here. Studies in highly contaminated environments are summarised in Table 18 and those in common indoor environments in Table 19. Only epidemiological

Table 18. Effects of short-term exposure in epidemiological studies of workers exposed to highly contaminated environments.

NOEL (spores/m^3)	LOEL (spores/m^3)	Species	Study group	Effects	Comments	Reference
1×10^{5a}	3×10^{6a}	Mainly *Rhizopus*	29 wood trimmers	Decline in FEV_1 and FVC during 4 days and 3 months	Follow-up study, adjusted for individual factors by studying lung function change, only IgG antibodies to *Rhizopus* were detected, exposure measured by culture methods during all days.	Hedenstierna et al. (1985)
	1×10^{5a}	Mainly *Rhizopus* and *Paecilomyces*	28 wood trimmers	Decline in FEV_1 and MEF_{25} during 1 week	Adjusted for individual factors by studying lung function change, species were detected by culture, exposure measured on 1 day, bacteria were measured but not evaluated. Dust, endotoxin, and terpene levels were low.	Dahlqvist et al. (1992)
	1×10^{5b}	Various fungi	89 farmers	Eye irritation	Cross-sectional study, adjusted for confounders and other bioaerosol agents, acute symptoms and exposure recorded simultaneously.	Eduard et al. (2001)
2×10^5		Various fungi	25 municipal waste handlers	Change in inflammatory cells and mediators in induced sputum after 3 days of exposure	Cross-sectional study, qualitative evaluation of smoking and age, other bioaerosol agents were studied in separate models, exposure recorded during days between sputum inductions.	Heldal et al. (2003b)
	2×10^5	Various fungi	31 municipal waste handlers	Increased neutrophils in nasal lavage and nasal congestion after 3 days of exposure	Cross-sectional study, qualitative evaluation of smoking and age, other bioaerosol agents were studied in separate models, exposure recorded during days between nasal lavages.	Heldal et al. (2003a)
	3×10^5	Various fungi	22 municipal waste handlers	Cough	Cross-sectional study, qualitative evaluation of smoking and age, other bioaerosol agents were studied in separate models and were more weakly associated with cough, acute symptoms and exposure were recorded simultaneously.	Heldal et al. (2004)

Table 18. Continued on next page

Table 18. Continued.

NOEL (spores/m³)	LOEL (spores/m³)	Species	Study group	Effects	Comments	Reference
6×10^5		Mainly *Rhizopus* and *Penicillium*	11 sawmill workers	Inflammatory markers in nasal lavage	Compared workers at work and on holiday, no adjustments for confounding, other bioaerosol agents were measured.	Roponen et al. (2002)
	3×10^{6b}	Various fungi	89 farmers	Cough, nasal irritation	Cross-sectional study, adjusted for confounders and other bioaerosol agents, acute symptoms and exposure were recorded simultaneously.	Eduard et al. (2001)
	1×10^8–4×10^8	Various fungi and actinomycetes	4 farmers with hypersensitivity pneumonis	Fever attacks	Cross-sectional study, no confounder adjustment, confirmed diagnosis, exposure was measured when the work causing a fever attack was repeated.	Malmberg et al. (1993)
	2×10^9	Various fungi and actinomycetes	6 farmers with ODTS	Fever attacks		

[a]Estimated from culture counts by multiplying with a factor of 10.
[b]Calculated to equal an 8-hour exposure if exposure time was less than 8 hours by multiplying with the weight factor 8 hours/exposure time.
FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; Ig: Immunoglobulin; LOEL: lowest observed effect level; MEF_{25} maximum expiratory flow at 25% of FVC; NOEL: no observed effect level; ODTS: organic dust toxic syndrome.

studies fulfilling the inclusion criteria were included, i.e., exposure assessment based on personal sampling, objective outcomes, or self-reported symptoms if adjusted for confounders. The criteria were applied less stringent for studies of populations exposed to common indoor levels, as few studies were found. cfu counts were multiplied by 10 in order to estimate spore counts (Sections 5 and 11).

A study of wood trimmers showed a NOEL of 1×10^5 spores/m³ and a LOEL of 3×10^6 spores for a 4-day change in FVC (Hedenstierna et al., 1986) (Table 18). Exposure to fungi was measured by culture. In the group with highest exposure, a decline in FVC was observed during 3 months preceding the exposure measurements. It seems unlikely, however, that exposure levels were very different from those that had been measured during the 4-day period (Section 11.4.2). In another study of Swedish wood trimmers, a LOEL of 1×10^5 spores/m³ for a cross-week change in FEV_1 and MEF_{25} was found (Dahlquist et al., 1992).

LOELs for nasal congestion and inflammatory markers in the nose of waste handlers were found at 2×10^5 spores/m³ (Heldal et al., 2003a) (Table 18). A study of sawmill workers suggested a NOEL at a higher level, 6×10^5 spores/m³, but this study was very small ($N=11$; Roponen et al., 2002). Inflammatory markers in induced sputum of the population of waste handlers showed a NOEL at 2×10^5 spores/m³ (Heldal et al., 2003b).

LOELs for cough in waste handlers (Heldal & Eduard, 2004) and in farmers (Eduard et al., 2001) were 3×10^5 and 3×10^6 spores/m³, respectively (Table 18). In farmers, LOELs of 1×10^5 spores/m³ for eye irritation and 3×10^6 spores/m³ for nasal irritation were found (Eduard et al., 2001). For fever, LOELs of 1×10^8 to 4×10^8 spores/m³ were observed in cases of hypersensitivity pneumonitis, and 2×10^9 spores/m³ in cases of ODTS (Malmberg et al., 1993) (Table 18).

Thus, at exposure levels of 1×10^5 spores of various fungi/m³, both objective findings as well as symptoms emerge in populations working in highly contaminated environments. The findings in wood workers seem most reliable, as fungal spores are often the dominating agent besides wood dust. Studies in wood trimmers are particularly interesting as little wood dust is generated in the sorting and trimming department. Dust, endotoxin, and terpene levels in trimming departments of Swedish sawmills have been shown to be low (Dahlquist et al., 1992) and low exposure levels to endotoxins (GM approximately 16 EU/m³) was also reported in a study of Finish sawmill workers (Roponen et al., 2002). Bacterial levels were similar to fungal levels in the Swedish study by Dahlquist et al. (1992). However, the counting of bacteria by fluorescence microscopy has been shown to have poor accuracy (Eduard et al., 2001) and there is little information on risk levels for bacteria.

Only one study of health effects related to short-term exposure in a population exposed to common indoor levels was found (Roponen et al., 2003) (Table 19). This well-designed study of inflammatory markers in nasal lavage, and irritation and systemic symptoms in schoolteachers, indicated NOELs of 7×10^2 spores/m³, including exposure in the work and home environment.

12.3. Effects related to long-term exposure

Cross-sectional studies of lung function and respiratory symptoms are considered here where the effect registration

Table 19. NOELs and LOELs observed in epidemiological studies of populations exposed to common indoor environments.

NOEL (spores/m^3)	LOEL (spores/m^3)	Species	Study group	Effects	Comments	Reference
	1	*Alternaria*	107 symptomatic and 107 nonsymptomatic office workers	Work-related respiratory symptoms.	Matching on age, gender, and atopy. Adjusted for smoking, house dust mite allergen in floor dust and indoor/outdoor humidity. Not clear if personal sampling was applied. No data on other fungi, total fungi, and the number of collected samples were given.	Menzies et al. (1998)
$7\times10^{2a,b}$		Various fungi	41 teachers	No changes in nasal lavage: NO, IL-4, IL-5, IL-6, and TNFα. No nasal, eye, throat irritation, lower airways symptoms, and nonspecific symptoms.	Population randomly selected. Personal exposure measurements at work and at home. Exposure to bacteria was also studied. Symptoms of the last week were recorded and nasal lavages were done at the end of the sampling period. Confounder assessment by separate analyses in asthmatic and smoking sub-groups.	Roponen et al. (2003)
	1×10^{4a}	Various fungi	264 day-care workers from 28 centres	Nasal discharge and congestion.	Day-care centres randomly selected.	Li et al. (1997)
	3×10^{2a}	*Aspergillus fumigatus*		Nasal congestion, cough and phlegm, lethargy, fatigue.	Stationary measurements and number of measurements not given. Exposure variables not specified in models. Adjusted for age, gender, and education. Separate models with other fungal species, total culturable bacteria, and house dust allergens on settled dust did not show associations.	
	1×10^{4a}	Various fungi (50% *Sporobolomyces salmonicolor*)	14 hospital workers with asthma ($n=4$) and rhinitis ($n=11$)	Asthma and rhinitis symptoms.	IgG positive but skin prick test negative to *S. salmonicolor*. Positive provocation tests with extract of *S. salmonicolor*. Stationary measurements at 10 locations. Number of measurements not given.	Seuri et al. (2000)

[a]Estimated from culture counts by multiplying with a factor of 10.

[b] Including exposure in the work and the home environment.

IL: interleukin, Ig: immunoglobulin, LOEL: lowest observed effect level, NO: nitric oxide, NOEL: no observed effect level, TNFα: tumour necrosis factor alpha.

is not linked individually to the day(s) that exposure was measured. The measured exposure is therefore expected to represent an average level over a certain time period. Studies in common indoor environments are summarised in Table 19 and those in highly contaminated environments in Table 20. The inclusion criteria used in Section 12.2.3 apply also here.

Increased albumin, fibronectin, and hyaluronan levels in bronchoalveolar lavage of 19 wood trimmers indicated low intensity inflammation compared to a control group of 25 healthy subjects without occupational exposure to fungi (all nonsmokers) (Johard et al., 1992). The exposure level indicated a LOEL of 1×10^5 spores/m^3. The design was cross-sectional but no information on the exposure period was

Table 20. Effects of long-term exposure in epidemiological studies of workers exposed to highly contaminated environments.

NOEL (spores/m^3)	LOEL (spores/m^3)	Species	Study group	Effects	Comments	Reference
	1×10^5	Various fungi	19 wood trimmers and 25 controls	Inflammatory markers in bronchoalveolar lavage	All nonsmokers. No adjustments for confounding or bacteria (that was measured).	Johard et al. (1992)
1×10^5		Various fungi	19 wood trimmers and 25 controls	FEV_1 and FVC	As above.	Johard et al. (1992)
	1×10^{5a}	Various fungi	82 joinery workers	Phlegm	Cross-sectional study, adjustments for confounders, other bioaerosol agents studied in separate models were more weakly associated with the effect and were not strongly correlated with fungal exposure. Exposure was measured by culture methods.	Alwis et al. (1999)
	3×10^{5a}	Various fungi	82 joinery workers and 108 saw-/chip mill workers	Throat irritation	As above.	Alwis et al. (1999)
	4×10^{5a}	Various fungi	108 sawmill and chip mill workers	Breathlessness	As above.	Alwis et al. (1999)
	2×10^6	Various fungi (42% *Rhizopus microsporus*)	107 wood trimmers	Morning cough	Cross-sectional study, adjusted for confounders and wood dust. Lowest exposure 4×10^5 spores/m^3.	Eduard et al. (1994)
	1×10^7	*Penicillium glabrum*	648 cork workers	Hypersensitivity pneumonitis, X-ray changes; restrictive lung function related to years employed	Large cross-sectional study, adjustments for smoking (only X-ray changes). Prevalences of the outcomes were 4%, 57%, and 30%, respectively. No information on previous exposure.	Ávila & Lacey (1974)
	1×10^7	Various fungi	1614 farmers	Nonatopic asthma	Large cross-sectional study, adjustments for confounders and endotoxins (correlated with fungi). Exposure was estimated with considerable random error.	Eduard et al. (2004)

[a]Estimated from culture counts by multiplying with a factor of 10.

FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; IL: interleukin; LOEL: lowest observed effect level; NOEL: no observed effect level.

provided. It is therefore not clear whether these differences are effects of short- or long-term exposure.

In a cross-sectional study of sawmill, joinery, and chip mill workers LOELs for respiratory symptoms such as phlegm, throat irritation, and breathlessness ranged from 1×10^5 to 4×10^5 spores/m^3 (Alwis et al., 1999). In another cross-sectional study of wood trimmers, morning cough indicated a LOEL of 2×10^6 spores/m^3 (Eduard et al., 1994). This is probably a high estimate because the population did not include individuals exposed below 4×10^5 spores/m^3.

Exposure levels that correlated with X-ray abnormalities in cork workers were approximately 10^7 spores/m^3 (Ávila & Lacey, 1974). As hypersensitivity pneumonitis also was present in this population, with a prevalence of 4%, a similar LOEL is indicated for this outcome as well. Restrictive lung function was correlated with duration of employment of the cork workers, indicating an association with cumulative exposure at the level of 10^7 spores/m^3. There was, however, limited information and uncertainty about previous exposure levels in this study.

In farmers, a LOEL of 10^7 spores/m^3 was found for asthma in nonatopic farmers (Eduard et al., 2004) but this level may have been attenuated by substantial random errors in the estimated exposure.

Thus, both objective findings as well as respiratory symptoms begin to appear at exposure levels of $\geq1\times10^5$ spores of various fungi/m^3 in populations working in highly contaminated environments. In cork workers, hypersensitivity pneumonitis, X-ray changes, and restrictive lung function were observed at an exposure level of 1×10^7 spores/m^3, mainly from *Penicillium glabrum.*

In an epidemiological study of a fairly large population ($n=264$) of randomly selected day-care centres exposed to common indoor levels, a LOEL of 10^4 spores/m^3 of various fungi for nasal symptoms was found (Li et al., 1997).

A small cluster of asthma and rhinitis cases in a small population of hospital employees working in a water-damaged building had positive bronchial challenge tests to *Sporobolomyces salmonicolor*. Exposure levels were approximately 10^4 spores/m^3 (50% *S. salmonicolor*) in these employees and could indicate a LOEL for asthma and rhinitis symptoms in sensitive individuals. However, exposure was poorly described and asthma and rhinitis was probably induced at higher exposure levels (Seuri et al., 2000). In a case-control study, work-related respiratory symptoms were associated with approximately 10 *Alternaria* spores/m^3. No exposure levels to total fungi or other species were reported (Menzies et al., 1998).

These studies in common indoor environments indicate lower effect levels than the studies of highly exposed populations. However, the workers in the water-damaged hospital had asthma or rhinitis and represent a sensitive group (Seuri et al., 2000). Furthermore, these LOELs are similar to the LOELs found by bronchial provocation in a susceptible group of asthmatic patients allergic to the fungi used in the challenge, which is not very likely since LOELs are expected to be higher in healthy subjects. The exposure assessment in the former studies was insufficient due to stationary sampling, little information on sampling strategy and few or an unknown number of measurements. There is therefore insufficient support for the assumption that health effects can be induced by fungal spores in healthy workers in common indoor environments at lower exposure levels than in highly contaminated environments.

13. Previous evaluations by national and international bodies

NEG evaluated microorganisms in 1991 but did not find a scientific basis for criteria or guidelines for fungal spores (Malmberg, 1991).

The Russian Federation is the only nation that has adopted official occupational exposure limits (OELs) for microorganisms. The Russian maximum allowable concentrations are given for specific fungi and actinomycetes (State Committee for Hygiene and Epidemiological Surveillance, 1993). These limits appear to be based on allergenicity in animal models. The list is shown in Table 21. The limits range from 10^3 to 10^4 cfu/m^3, which may correspond to 10^4 to 10^5 spores/m^3. However, many of the listed species are used for production of food and antibiotics and probably have high viability when freshly cultivated. A factor of 10 for conversion of cfu to spores may therefore be too high. The species are classified according to hazard and allergenicity but no information is provided on criteria used for classification.

The current opinion of the American Conference of Governmental Industrial Hygienists (ACGIH) is that the establishment of threshold limit values (TLVs) for total culturable or countable bioaerosols is not possible (ACGIH, 2006). Their most important arguments are (1) that bioaerosols are generally complex mixtures of many agents, (2) the diversity of responses to microorganisms, and (3) insufficient information on exposure-response relationships.

Rao et al. (1996) reviewed quantitative standards and guidelines for airborne fungi and found that these recommendations were primarily based on the low exposure levels in "normal" environments given either as absolute or relative levels by comparing to outdoor levels, and not on health effects data. A number of guidance documents have been published, e.g., by the ACGIH (1999), AIHA (1993), Health Canada (2004), Institute of Medicine in the United States (2004), and WHO (2009). Many of these guidelines contain recommendations on prevention because dampness-related symptoms can be remediated by reducing humidity without knowledge of the causative agent.

14. Evaluation of human health risks

14.1. Assessment of health risks

14.1.1. Airway and lung inflammation

Only one animal study provides relevant information on effects of single exposure (Fogelmark et al., 1991). This study showed no lung inflammation after exposure for 4 hours to 3×10^7 spores/m^3 of *Aspergillus fumigatus* and three other

Table 21. Maximum allowable concentrations of fungi and actinomycetes issued in the Russian Federation.

Microorganism	Maximum allowable concentration, cfu/m^3	Hazard class[a]	Allergenicity
Fungi			
Acremonium chrysogenum	5×10^3	III	+
Ampelomyces quisqualis	10^4	III	
Blakeslea trispora	10^4	III	+
Candida scotti	10^3	II	
Candida tropicalis	10^3	II	
Candida utilis	10^3	II	
Candida valida	10^3	II	
Cryptococcus laurentii var. *magnus*	0.5 mg/m^3	II	+
Fusidium coccineum	5×10^3	III	
Penicillium canescens	2×10^3	III	
Saccharomyces cerevisae	0.5 mg/m^3	II	+
Actinomycetes			
Actinomyces roseolus	10^3	II	
Streptomyces aureofaciens	5×10^3	III	+
Streptomyces erythreus	3×10^3	III	+
Streptomyces lactis	10^4	III	
Streptomyces kanemyceticus	5×10^3	III	+
Streptomyces rimosus	3×10^3	III	+

Note. Translated from Russian by Dr Natalia Romanova (Northwest Public Health Research Centre, St. Petersburg, Russia).
[a]No information provided on the hazard classification.

fungal species. However, neutrophil and eosinophil counts increased in bronchoalveolar lavage after a single exposure to 3×10^8 spores/m^3 of the actinomycete *Saccharopolyspora rectivirgula*. The high incidence of ODTS attacks among college students who participated in a party in a room where the floor was covered with mouldy straw (Section 11.3; Brinton, 1987) indicates that acute reactions do occur. Furthermore, Kotimaa et al. (1990) reported exposure levels up to 1.2×10^7 cfu/m^3 during straw handling on farms, indicating that exposure levels of 10^8 spores/m^3 are not unlikely.

Lung inflammation was induced by all tested fungal and actinomycete species when exposures were repeated daily for 5 weeks in the animal study described above. The lowest LOEL was 4×10^5 spores/m^3 for *Aspergillus fumigatus* when calculated to equal an 8-hour exposure (Fogelmark et al., 1991).

Four epidemiological studies in waste handlers (Heldal et al., 2003a, 2003b), sawmill workers (Roponen et al., 2002), and wood trimmers (Johard et al., 1992) indicate NOELs at 2×10^5 to 6×10^5 spores/m^3 and LOELs at somewhat lower levels 1×10^5 to 2×10^5 spores/m^3. The study of waste handlers has the strongest design, as exposure was measured on 3 days between nasal lavages and sputum inductions. However, inflammatory markers and cells in induced sputum showed no association with fungal spores, indicating a NOEL of 2×10^5 spores/m^3, whereas nasal lavage showed a LOEL of 2×10^5 spores/m^3 for increased neutrophil counts. The effects of exposure to endotoxins, glucans, and bacteria, and the potential confounders smoking, age, and atopy were evaluated qualitatively and could not explain the associations with fungal exposure. All studies were relatively small, however, and there is a considerable risk of false-negative results. Furthermore, as methods for sputum induction and nasal lavage were recently developed, little is known about the development of inflammation markers over time, which complicates interpretation of the results.

14.1.2. Respiratory function

Two human challenge studies present information on effect levels regarding respiratory function. In a single-dose study of school employees with sick building syndrome, no changes in mucosal and systemic symptoms, lung function, and blood leukocytes were found after challenge with 8×10^3 *Penicillium chrysogenum* spores/m^3 or 4×10^3 *Trichoderma harzianum* spores/m^3 (Meyer et al., 2005). In the other study, asthmatic patients were exposed to successively increasing doses, and a reduced airway conductance was found after challenge with 1×10^4 *Penicillium* sp. spores/m^3 and 2×10^4 *Alternaria alternata* spores/m^3 (Licorish et al., 1985). The patients had specific IgE to the fungi used in the test, whereas sensitisation of the school employees was not clear (positive basophil histamine-release tests but negative skin prick tests and RASTs).

In a Swedish study, wood trimmers exposed to 1×10^5 spores/m^3 showed no changes in FVC and FEV_1 after 4 days and 3 months, respectively (Hedenstierna et al., 1986). In the same study, wood trimmers from another sawmill exposed to 3×10^6 spores/m^3 showed FVC and FEV_1 declines after 4 days with exposure measurements. Both FVC and FEV_1 had also declined during 3 months preceding the exposure measurements. It seems unlikely that exposure levels during these months were very different from those that had been measured previously (Section 11.4.2).

An exposure-response association for changes in FVC and MEF_{25} after 3–5 days exposure to a median of 1×10^5 spores/m^3 was found in another study of Swedish wood

trimmers (Dahlquist et al., 1992). A LOEL of 2×10^5 spores/m^3 was found for increased nasal congestion after 3 days of exposure among waste handlers (Heldal et al., 2003a). In cork workers exposed to a mean level of approximately 10^7 spores/m^3, exposure duration was associated with restrictive lung function (Ávila & Lacey, 1974). However, limited information on current and previous exposure levels and outcomes complicates interpretation of the latter study.

The two Swedish studies of wood trimmers reported different effect levels. A NOEL of 1×10^5 spores/m^3 for short-term FVC and FEV_1 decline in wood trimmers may be based on the study by Hedenstierna et al. (1986), whereas Dahlquist et al. (1992) report an exposure-response association at the same level for FEV_1 and MEF_{25} decline during a week. This may indicate that effects start to appear at exposure to 1×10^5 spores/m^3. The estimate in the latter study is more reliable as fungal spores were counted by scanning electron microscopy (SEM). This LOEL is consistent with NOELs of 4×10^3 to 8×10^3 spores/m^3 observed in a single-dose human challenge study (Meyer et al., 2005), and with the LOELs of 1×10^4 to 2×10^4 spores/m^3 found in the human challenge study of asthmatic patients (Licorish et al., 1985), since LOELs are expected to be higher in healthy subjects. The LOEL for short-term increase in nasal congestion in waste handlers of 2×10^5 spores/m^3 (Heldal et al., 2003a) is also in the same region.

14.1.3. Respiratory symptoms

No respiratory and general symptoms were observed in the human challenge study of school employees after challenge with 8×10^3 *Penicillium chrysogenum* spores/m^3 or 4×10^3 *Trichoderma harzianum* spores/m^3 calculated to equal an 8-hour exposure (nasal and throat irritation, nasal congestion, and headache were examined) (Meyer et al., 2005). LOELs of 1×10^5 to 4×10^5 spores/m^3 for short-term and long-term respiratory symptoms such as cough, eye irritation, throat irritation, phlegm, and bronchitis were observed in three epidemiological studies of farmers, waste handlers, and joinery, sawmill, and chip mill workers (Alwis et al., 1999; Eduard et al., 2001; Heldal & Eduard, 2004). Cough in wood trimmers and farmers, and nasal irritation in farmers indicated higher LOELs of 2×10^6 to 3×10^6 spores/m^3 (Eduard et al., 1994, 2001). However, the wood trimmer population did not contain workers exposed to less than 4×10^5 spores/m^3, which precluded the exploration of symptoms at lower exposure levels. The LOEL of work-related cough in the farmers study seems genuine as exposure to other bioaerosol components was measured but these agents were not associated with cough and the internal reference group was exposed to 2×10^3 to 2×10^4 spores/m^3 (Eduard et al., 2001). In this study, the risk of nasal irritation was already high at an exposure level of 1×10^5 spores/m^3 although not significantly (OR 4.1, 95% CI 0.88–19).

Thus, most associations with respiratory symptoms indicate LOELs of 1×10^5 to 4×10^5 spores/m^3, which is similar to the LOELs indicated for short-term respiratory function decline. However, associations with cough and possibly nasal irritation in farmers indicate higher LOELs of 2×10^6 to 3×10^6 spores/m^3.

14.1.4. Asthma

Only one epidemiological study related the prevalence of current physician-diagnosed asthma in nonatopic farmers to exposure to fungal spores and other agents (Eduard et al., 2004). Annual exposure levels were estimated from task-based measurements, which is likely to have introduced substantial random error. The observed LOEL of 10^7 spores/m^3 is therefore most likely a high estimate. In addition, endotoxins and ammonia were also associated with asthma and could not be adjusted for due to high correlation between exposure measures.

In atopic farmers, an inverse exposure-response association was found (Eduard et al., 2004). Studies from different countries have shown that the occurrence of atopic diseases in farming populations is lower than in the general population, especially in children. This seemingly protective effect is associated with contact with farm animals, and it was postulated in the so-called hygiene hypothesis that exposure to microbial agents, especially endotoxins from Gram-negative bacteria, mediated this protective effect; review by Schaub et al. (2006). The results of the farmer study indicate that such a protective effect may also result from exposure to the farm environment as an adult and that even exposure to fungal spores can be involved (Eduard et al., 2004). It is also possible, however, that farmers with allergic asthma change their production, methods, and/or tasks in order to reduce exposure, or even quit farming, which would select asthmatic farmers away from highly exposed farm work (healthy worker effect).

14.1.5. Hypersensitivity pneumonitis and organic dust toxic syndrome

Single-dose human challenge studies of patients with hypersensitivity pneumonitis found LOELs for typical attacks of the disease at 2×10^6 *Pleurotus ostreatus* spores/m^3 and 6×10^6 *Lentinus edodes* spores/m^3, calculated to equal an 8-hour exposure, respectively (Cox et al., 1988, 1989). These LOELs are lower than the levels that induced fever attacks in farmers with a clinically confirmed diagnosis of the disease, 1×10^8 to 4×10^8 spores/m^3 (Malmberg et al., 1993). The farmer study also indicates that fever attacks in farmers with ODTS develop at approximately 10 times higher exposure levels (2×10^9 spores/m^3) than in farmers with hypersensitivity pneumonitis(Malmberg et al., 1993). Further information is obtained from a study of cork workers exposed to 1×10^7 spores/m^3 (mainly *Penicillium glabrum*) where 4% of the population had hypersensitivity pneumonitis. X-ray changes, a typical observation in hypersensitivity pneumonitis patients, had a prevalence of 57% and correlated with current exposure levels (Ávila & Lacey, 1974).

These data indicate that attacks of hypersensitivity pneumonitis may develop after exposure to 2×10^6 to 6×10^6 spores/m^3 of a single species, and that the disease can develop after long-term exposure to 1×10^7 spores/m^3. Higher LOELs were

reported in the farmer study (1×10^8 to 4×10^8 spores/m^3). However, farmers were exposed to spores from various species. As hypersensitivity pneumonitis seems to be species specific (Section 11.2.1), the exposure level of the species that the farmers were sensitised to is likely to be lower. It cannot be ruled out, however, that the potential to induce hypersensitivity pneumonitis differs between species.

14.1.6. Studies in common indoor environments

The evidence from epidemiological studies of populations exposed to common indoor air is insufficient for conclusions about effect levels to be drawn, except for one well-designed study (Roponen et al., 2003) indicating a NOEL for inflammation in the nose and respiratory symptoms of 7×10^2 spores/m^3, including exposure at home and at work (Sections 12.2 and 12.3).

14.1.7. The role of specific organisms

The animal study by Fogelmark et al. (1991) shows that all tested species are capable of inducing inflammation in the airways, but differ in inflammatory potential. This is further documented in many other animal studies (Section 10.9). The limited number of human challenge studies with intact spores provide limited information on species differences. A few epidemiological studies of long-term effects have analysed the role of specific fungi (Section 11.5.2). Stronger associations have been found for *Aspergillus fumigatus* in a waste handler study (Coenen et al., 1997) and for *Aspergillus* and *Alternaria* sp. in common indoor air studies (Li et al., 1997; Menzies et al., 1998). However, these studies suffer from weaknesses in the exposure assessment. The extremely low LOEL of 1 *Alternaria* spores/m^3 in the study by Menzies et al. (1998) is highly unlikely considering the challenge study by Licorish et al. (1985) where a LOEL of 2×10^4 spores/m^3 was found in asthmatic patients allergic to this fungus. The implicated species in these epidemiological studies represented only a fraction of the total number of fungi. In other studies, a single species dominated, e.g., *Penicillium glabrum* in a study of cork workers (Ávila & Lacey, 1974), *Rhizopus microsporus* in a study of wood trimmers (Eduard et al., 1994), and *Sporobolomyces salmonicolor* in a common indoor air study (Seuri et al., 2000). These studies do not indicate lower LOELs for the dominating species than observed in studies where species characterisation was not performed.

Little information is available about actinomycetes. The animal study by Fogelmark et al. (1991) indicated that a 10 times higher exposure level of spores of the actinomycete *Saccharopolyspora rectivirgula* induced inflammation in lung tissue of similar intensity as spores of the fungus *Rhizopus stolonifer* after prolonged exposure (Table 10). However, after a single exposure, only *S. rectivirgula* and none of the fungal species induced inflammation. Human challenge studies have further documented that *Saccharopolyspora rectivirgula* extracts can induce attacks of hypersensitivity pneumonitis and asthma in farmers' lung patients (Tables 11 and 12; Pepys & Jenkins, 1965; Edwards & Davies, 1981). In vitro and in vivo studies further document that spores from different actinomycetes species can induce airway and lung inflammation similarly to fungal spores. It is therefore reasonable to assume that the health effects following exposure to actinomycete spores do not differ qualitatively from fungal spores, but there is limited information on effect levels.

14.2. Groups at extra risk

Patients with hypersensitivity pneumonitis respond to fungal exposure with febrile attacks at a lower level than individuals without the disease. However, the exposure levels that induce typical attacks of the disease are relatively high, $\geq2\times10^6$ spores/m^3.

Patients with allergic asthma to fungi may have an increased risk. LOELs of 1×10^4 to 2×10^4 spores/m^3 found for airway obstruction in allergic asthmatics are 1 order of magnitude lower than LOELs found in epidemiological studies of lung function in healthy subjects. Children should also be regarded as a vulnerable group because they have a higher prevalence of allergic asthma to fungi (review by Bush & Portnoy, 2001).

The role of atopy is less clear. In a farmer study, the asthma prevalence decreased with exposure to fungal spores and other agents in atopic subjects (Eduard et al., 2004). This may be due to selection, but also the hygiene hypothesis may explanain these findings.

No information was found on sex differences and immunodeficient people. However, fungal infections may be life-threatening in immunocompromised cancer patients undergoing treatment (Richardson, 2005).

14.3. Scientific basis for an occupational exposure limit

In vitro and in vivo studies have shown that a large number of fungal and actinomycete species are capable of inducing inflammatory effects in phagocytic cells and lung tissue. There is also evidence from these studies that the inflammatory potency depends on species, with the mycotoxin-producing *Stachybotrys chartarum* and the facultative pathogenic *Aspergillus fumigatus* showing the highest potencies. However, epidemiological studies of various respiratory outcomes indicate fairly similar effect levels when expressed in spores/m^3. Thus, the inflammatory potency of most species occurring in the work environment seems to be fairly similar. Furthermore, fungi with high potency have to constitute a substantial fraction of the fungal biota before a significant increase in inflammatory potential can be expected.

The response in subjects with hypersensitivity pneumonitis and asthma is species specific, as shown by human challenge studies using fungal extracts and whole spores. It cannot be derived from these findings if the allergenic potential differs between species because the exposure levels leading to allergy is not known. For example, *Cladosporium*, *Alternaria,* and *Aspergillus* species are most often involved in fungal allergy but dominate also in outdoor air (Lacey, 1981).

A series of studies including various fungal species suggest that respiratory symptoms, airway inflammation, and

lung function impairment begin to appear at exposure levels of approximately 10^5 spores/m^3.

The lung function studies in wood trimmers are most interesting because the effect is objectively measured and wood trimmers are primarily exposed to fungal spores besides low levels of wood dust, bacteria, endotoxins, and terpenes. The effect of agents other than fungal spores is therefore limited. The effect of confounders was evaluated separately (smoking) or was eliminated by the follow-up design (age, height, and body weight). The study by Dahlquist et al. (1992) is preferred, as fungal spores were measured by microscopic counting. A relationship was found between exposure to fungal spores and FEV_1 decline in a population exposed to a median of 1×10^5 spores/m^3. This level is also compatible with NOELs of 4×10^3 to 8×10^3 spores/m^3 (calculated to equal an 8-hour exposure) observed in the human challenge study in which subjects inhaled a single concentration during 6 minutes (Meyer et al., 2005), and with LOEL of 1×10^4 to 2×10^4 spores/m^3 (calculated to equal an 8-hour exposure) found in the human challenge study where asthmatic patients inhaled graded doses by single inhalations (Licorish et al., 1985).

An OEL based on the study by Dahlquist et al. (1992) is likely to be representative for range of fungal species as studies of other populations suggest LOELs of similar magnitude. However, such a limit is not applicable if spores from mycotoxin-producing and/or opportunistic pathogenic species are prevalent.

As the fungal spore is the unit of interest in this document, exposure assessments should be based on nonculture methods. Microscopical methods seem most suitable at present. Characterisation of the fungal biota can be achieved by cultivation and by molecular biological methods. However, the presence of other airborne fungal particles as hyphae and fungal fragments have been demonstrated (Sections 3.3 and 3.6), but their role in respiratory disease is not clear as no animal or epidemiological studies addressing these particles have been published. In vitro studies suggest that the response to hyphae is different from the response to spores, but the studies have only been performed with pathogenic fungi (Section 8.1).

In conclusion, a LOEL of 10^5 spores/m^3 seems appropriate as a basis for an OEL for spores from diverse fungi. This LOEL is probably too high if spores from mycotoxin-producing and/or opportunistic pathogenic species are prevalent. However, there are not sufficient data to support LOELs for spores from such species. Individuals with asthma and sensitised to fungal allergens are more sensitive than working populations in general. The available evidence suggests a safety factor of 10.

14.4. Evaluations in common indoor environments

Very few studies of acceptable quality have been found of populations exposed to common indoor environments. However, the airborne fungal biota found in such environments suggest that effect levels found in studies of highly exposed populations can be applied to common indoor populations as well. Furthermore, in a challenge study of individuals with sick building syndrome NOELs of 4×10^3 to 8×10^3 spores/m^3 were found (Meyer et al., 2005). This NOEL is consistent with effect levels in highly contaminated environments since only a single dose level was applied in the human challenge study and higher NOELs are not precluded.

It is now generally accepted that dampness in buildings is related to respiratory effects, and the measurement of airborne fungal spores is just one of several ways to detect dampness (Bornehag et al., 2001). Several strategies are available for the evaluation of "mould problems" and many criteria have been proposed to evaluate common indoor environments (Rao et al., 1996; WHO, 2009) (Section 13). These criteria specify much lower levels of airborne fungi than the LOELs found in this review, but the criteria are only indirectly related to health effects as they have been proposed to identify "sick buildings".

15. Research needs

More information is needed on NOELs of exposure to spores from specific fungi, including mycotoxin-producing and opportunistic pathogenic species. This requires animal studies as human challenge with whole spores is generally regarded unethical (Section 11.2.1). Studies should be carried out with aerosol exposure over extended periods.

More data from large longitudinal studies with good quality exposure assessment of fungal spores are needed because, except for hypersensitivity pneumonitis and ODTS, it is not known whether fungal spores cause new-onset disease or aggravates existing respiratory conditions. The reviewed occupational epidemiological data are only from cross-sectional studies.

Molecular biological methods seem well suited for non–culture-based assessment of fungi. Development of quantitative methods for the measurement of specific organisms and genera is therefore needed to improve the exposure assessment in future studies. Even chemical markers of fungi can be considered, especially agents shown to have inflammatory properties such as β(1→3)-glucans (Douwes, 2002).

The few occupational studies that have examined allergic outcomes show contradictory results. In some highly exposed populations, sensitisation to prevalent fungal species is almost absent, whereas fungal allergy is not uncommon in the general population and in working populations exposed to fungal enzymes (Sections 3.5 and 11.2.2). There is some information in the experimental studies that deserves more attention, however. In animal studies, viable spores induced allergic responses in contrast to nonviable spores, and repeated-exposure studies also demonstrated eosinophilic inflammation in addition to neutrophils and macrophages (Section 10.9). Mechanistic studies have shown that dendritic cells prime a nonallergic response to spores and an allergic response to hyphae (Section 8.1). The allergic response to viable spores and to hyphae can be due to the production of allergens by germinating spores and growing

hyphae (Section 3.5). Furthermore, Sercombe et al. recently demonstrated that germinating spores in the nasal cavity of healthy subjects were common (Sercombe et al., 2006). It is therefore important to develop methods for quantification of viable and nonviable spores and hyphae and apply these in epidemiological studies.

The role of the small but probably numerous fungal fragments needs to be clarified. Experimental in vivo and in vitro studies may provide toxicological data. Furthermore, epidemiological studies are needed that require the development of a method for measurement of these particles in diverse aerosols.

Abbreviations and acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
AED	aerodynamic diameter
bw	body weight
CAMNEA	collection of airborne microorganisms on nucleopore filters, estimation and analysis
cfu	colony-forming units
CI	confidence interval
ECP	eosinophilic cationic protein
EU	endotoxin units
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
IFNγ	interferon gamma
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
LD_{50}	lethal dose for 50% of the animals at single exposure
LOEL	lowest observed effect level
MCP	monocyte chemoattractant protein
MEF_{25}	maximum expiratory flow at 25% of FVC
MIP	macrophage inflammatory protein
MVOC	microbial volatile organic compound
NEG	The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
NO	nitric oxide
NOEL	no observed effect level
ODTS	organic dust toxic syndrome
OEL	occupational exposure limit
OR	odds ratio
PaO_2	arterial oxygen tension
PMN	polymorphonuclear leukocytes (mainly neutrophils)
PPR	prevalence proportion ratio
RAST	radio-allergosorbent test
ROS	reactive oxygen species
SEM	scanning electron microscope
sp/spp	species (singular/plural)
TGFβ	transforming growth factor beta
Th cells	T helper cells
TLco	lung transfer factor for carbon monoxide
TLR	Toll-like receptor
TLV	threshold limit value
TNFα	tumour necrosis factor alpha

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Data bases used in the search for literature

A literature search was conducted in the Medline, Toxline, Arbline, OSH-ROM, and ISI bases as well as the author's own database. Further references were obtained from reviewed papers. The last search was performed in November 2006. The following terms were used in the literature search:

Agent
Fungal spores
Fungi
Moulds/molds
Hyphae
Actinomycetes
Fungal components
Mycotoxins
Fungal antigens
Fungal allergens
Glucans
Fungal antibodies
Antibodies
Fungal IgG antibodies
Fungal IgG
Fungal IgE antibodies

Fungal IgE
Effects
Toxicity
In vitro
In vivo
Animal
Cytotoxicity
Mutagenicity
Carcinogenicity
Teratogenicity
Reproductive effects
Human challenge studies
Provocation tests
Exposure-response
Exposure-effect
Dose-response
Dose-effect
Respiratory effects
Inflammation
Lung function
Airway disease
Allergic alveolitis
Hypersensitivity pneumonitis
Asthma
Allergy
Chronic bronchitis
Fever
Epidemiology
Occupational environments
Agriculture
Farmers
Mushroom cultivation
Sawmills
Wood industry
Breweries
Cheese production
Indoor air, built environment, schools
Municipal waste
Sugar mills

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